

HORTICULTURAL APPLICATIONS OF LEDS WITH LETTUCE AND TOMATO
AND TRAINING THE NEXT GENERATION OF SCIENTISTS

A Thesis

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by

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ABSTRACT

LEDs have varied applications in horticulture with both food and floriculture crops. The current study investigated their use with both lettuce in the greenhouse and tomato in growth chambers. Lettuce work focused primarily on electrical efficacy of LEDs when compared with traditional HPS lights and their effect on 13 different varieties of lettuce. It was found that LEDs were, on average, twice as productive in terms of edible biomass produced per kWh used. Some photomorphological effects were observed in height and width characteristics after further investigation. Tomato growth focused on the effects of varying ratios of red:blue light on flower number, developing fruit, seedling height, and ascorbic acid concentration, and found significant effects in all categories except ascorbic acid production. The educational component of this thesis springs from the development of a curriculum supplement for 6th to 8th graders, following the Next Generation Science Standards (NGSS), using Plant Science as a basis for scientific inquiry.

BIOGRAPHICAL SKETCH

Erica Hernandez was born in Houston, Texas, and moved to Portland, Oregon, where she spent most of her first 21 years. She eventually discovered an interest in science and plants and moved to Arizona to pursue that passion at the University of Arizona.

Through opportunities offered there, she participated in the Arizona Space Grant Consortium as a Space Grant Intern on the Lunar Greenhouse Project under Dr. Gene

Giacomelli. She received her Bachelor's of Science in Plant Science (minor in Computer Science) in May 2016, after a long 7 years of undergraduate work, filled with internships and hands-on plant cultivation and research experience. She was accepted to Cornell University in 2016 and worked with Dr. Neil Mattson in her Master's program on horticultural applications of LED lights in greenhouses and growth chambers, and hopes that her future is not *too* full of lettuce.

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CHAPTER 1

QUALITY, YIELD, AND BIOMASS EFFICACY OF SEVERAL HYDROPONIC LETTUCE (*LACTUCA SATIVA* L.) CULTIVARS IN RESPONSE TO HIGH PRESSURE SODIUM LIGHTS AND LIGHTING EMITTING DIODES FOR GREENHOUSE SUPPLEMENTAL LIGHTING

Abstract

Lettuce is an economically important crop that can be grown either in field or greenhouse. Different challenges are present in either environment; therefore cultivar selection is important. For hydroponic greenhouse lettuce there is relatively little published information on variety selection under different lighting sources. In experiment I, 25 varieties of lettuce were grown and sampled under HPS lights for six months. Qualitative observations were taken on size, sensitivity to tip burn, and bolting. Based on these observations thirteen varieties were selected for the second experiment. The objective of experiment II was to determine the influence of lighting using high pressure sodium (HPS) or light emitting diodes (LED) on plant fresh weight, height, tip burn index, bolting, and Brix. Experiment III was similar to experiment II but with fewer varieties (5) to allow for greater number of replicates per treatment per crop cycle. Each experiment was replicated over time so that there were three crop cycles per experiment. Light sources were controlled using an algorithm, Light and Shade System Implementation (LASSI), to achieve a constant average daily light integral under each treatment and crop cycle. Electrical consumption and efficacy (fresh weight per kWh) from each treatment was estimated using data collected on power consumption from

representative lamps multiplied by the number of fixtures and the hours fixtures were on per crop cycle.

In experiment II, the fresh weight of 2 to 3 varieties was greater under HPS and 1 to 2 varieties under LED, depending on production cycle. HPS grown lettuce tended to have more tip burn and bolting in crop cycle 1 and 2, with cycle 3 showing similar tip burn incidence. Bolting was only consistently observed in one variety, Teodore. For the second cycle of experiment II a sensory test was conducted. There were clear differences between sweet and bitter varieties, as well as soft and crisp/crunchy varieties but few differences between lighting treatments within a variety. The LED array used less than half as much electricity as the HPS array, while producing relatively similar size lettuce therefore leading to electrical efficacies two to three times higher in LED than in HPS treatments. In experiment III, significant differences in height were found in Greenstar and Xandra, with HPS being larger than LED. Significant differences were also found in diameter in Greenstar, Xandra, Locarno, and Crunchita, with HPS again being larger than LED.

Introduction

Lettuce is a globally important crop, with a combined harvest of over 25 million tons in 2016 (FAOStat, 2016). This number has increased steadily since 1995 (FAOStat, 2016). Global production occurs on 1.25 million hectares of agricultural land. The United States is one of the top lettuce producers in the world, coming in second behind mainland China. Lettuce is the third most popular vegetable in the U.S. behind potatoes and tomatoes with an annual consumption of 25.5 pounds per capita per year (Bently,

2015). As a cool weather crop, lettuce has cooler air temperature requirements than some other popular greenhouse crops (Smith et al). Optimally, day temperatures should be around 23 C and night temperatures around 17 C. Bolting begins to be a problem as air temperatures rise and slowing of growth occurs with lower temperatures. Typical growth periods last anywhere from 65 to 80 days in the field, from seed to harvest, for full heads of lettuce during the summer, and up to 130 days during the winter. With this clear seasonal variation, greenhouse growing offers the benefits of stabilized climate and more consistent time to harvest. Beyond climate control, greenhouses also offer the ability to control lighting and shading conditions as well as CO₂ enrichment through the use of shading practices or the addition of supplemental lights (Both et al., 1998).

Lettuce is a facultative long-day plant, but previous studies have shown that precise photoperiod control may not be as crucial as with other crops (Bian, et al, 2016). 24-hour photoperiods induced an increase in phenolic compound concentration, while also reducing nitrate content in the lettuce at harvest time(Bian, 2016). However, certain varieties may be more susceptible to photoperiodic effects when it comes to bolting (Waycott, 1995), which becomes important when selecting varieties within a variety trial.

Daily light integral (DLI) is an important factor to control to achieve consistent growth rates for vegetable production. There is a direct relationship between the amount of light received and dry matter accumulation in lettuce, which makes DLI control relevant to greenhouse lettuce growers. Research into optimal DLI for lettuce production is well established, as much work has been done examining differing light levels. Both, et al determined that the optimal DLI for lettuce sits at 17 mol m⁻² d⁻¹

after examining 35 different treatments between 8 and 22 m² d⁻¹ (1997). LASSI (Lighting and Shade System Integration) is a computer algorithm to control DLI in the face of high variable environmental conditions (Albright, Both, & Chiu, 2000). LASSI factors in variables such as ambient light levels, cost structure of electricity (i.e. off-peak electricity), shade cloth status, time of day, a running sum of daily PPFD, light intensity that is provided by supplemental lights, dark period and overall photoperiod. This is dynamic lighting control, because the “the algorithm drew from no historical weather data and received no advance notice of the weather expected for the day.” (Albright, Both, & Chiu, 2000). Traditionally high intensity discharge (HID) fixtures such as HPS or metal halide (MH) have been used for supplemental lighting of lettuce in greenhouses. However, as LED cost decrease and efficacy increases, the adoption of LEDs has become more common.

Light Emitting Diodes (LEDs) are semiconductors that emit light when an electrical current passes through them. By varying the semiconductor materials used, different spectra (i.e. wavebands) can be obtained. The investigation into the use of LEDs for horticultural applications began in the late 1980s and early 1990s. While early studies were conducted with space-based missions in mind, applications on the ground have become more and more economically feasible as the technology progressed. These early studies involved red-only LEDs but have since developed to encompass a wide array of color capabilities (Morrow, 2008).

With the advancement of LED technology, there are some advantages of LEDs as compared to existing horticultural lights (HPS and metal halide). Examples include photosynthetically active radiation (PAR) efficacy (Wallace and Both 2016), the ability

to target specific wavebands/spectra of light, finer control over light intensity and periodicity of lighting (Davis & Burns, 2016), and a lower heat load produced in the direction of the light allowing them to be placed closer to a crop or operated during warm ambient conditions (Morrow, 2008; Ouzounis, 2015). When comparing potential for energy savings in LED over HPS lights the PAR efficacy, light output per unit electricity (units: $\mu\text{mol}/\text{j}$) is used (Both et al., 2017). However, PAR efficacy only tells us about fixture performance and does not account for plant performance under a lighting source. The biomass efficacy refers to the edible fresh weight or dry weight per unit electricity (units: g/kWh) under a given lighting fixture in a specific crop production environment.

Investigating how differing spectral environments can affect the morphology of different crops has been of interest for several decades (Stutte, 2009; Brown, 1995). Different morphological changes can be triggered by stimulating specific photoreceptors (Pocock, 2015). Plants have a variety of photoreceptors that are sensitive to different wavebands. Cryptochromes (blues) and phytochromes (reds) are the most common secondary receptors beyond chlorophyll (Massa, 2008). Under a higher ratio of blue to red light, plants tend to be more compact, with thicker cuticles and more intense red pigmentations whereas under little to no blue light, leaves tend to be broader, stalks tend to be taller, and there is less red pigment production (Massa, 2008). A higher ratio of far red to red light leads to shade avoidance behaviors such as etiolation, which is increased stem elongation (Massa, 2008).

A body of information has begun to develop on impacts of HPS vs. LED lighting (Martineau, et al, 2012). When grown under high pressure sodium lamps and LEDs of

the same intensities, the rate of net photosynthesis was not affected in lettuce (Ouzounis, 2015). Regarding morphological effects, under spectrums containing higher percentages of blue and UV light, lettuce heads tend to be more compact but slightly denser (Ouzounis, 2015). In red leaf lettuce, the red pigment anthocyanin increases in concentration with greater blue light exposure (Stutte, 2009). Leaf area and leaf expansion increases under increasing red light exposure (Ouzounis, 2015). Some work is currently being done to observe the effects of green light on lettuce and understand the biological mechanisms involved, but as of yet this system is still not completely understood (Kim et al, 2004).

Beyond yield and morphology, sensory properties (such as sweetness, bitterness, and texture) are important factors for selecting marketable varieties. Lettuce contains a large number of compounds that may contribute to variability of flavor, and therefore affect sensory taste panels. Taste testing is inherently subjective, but certain procedures can be undertaken to ensure consistency in evaluation (Taste Test Report). One quantitative measure associated with taste in fruits and vegetables is Brix. Brix (units: °Br) is a measure of soluble solids in fruits and juices. Brix analyses are easy to perform and often used by commercial producers and beyond. In selecting cultivars of lettuce and adopting new technology (such as LED lighting) it is also important to consider taste and texture.

Tipburn in lettuce manifests as a pattern of damage to the leaf margins, first appearing on interior, new leaves (Murdoch, 2003). Lettuce is sensitive to tip burn in a number of scenarios, though conditions affecting the uptake of calcium contribute the most. Rapid growth can outpace the ability of the plant to move calcium to developing

areas; high humidity conditions affect transpiration rates that also cut down on calcium transport rates; a lack of calcium in supplied fertilizer is most often not the cause (Frantz, et al., 2004). Leafy greens crops are variably affected by tip burn issues. For lettuce, potassium imbalances may also lead to the development of tip-burn (Inthichack, 2012). For such a complicated issue, control over environmental variables plays a key role in managing crop health.

While there has been previous research on the use of LEDs for lettuce, many research projects focus their efforts on a few carefully selected lettuce varieties in order to observe specific responses in those varieties. As such, there is not much directly comparable research between multiple varieties when looking at yield, morphology, and sensory analyses. Though previous research has shown that yield and morphology response to spectral quality in lettuce is highly variety dependent, it has not been shown whether those responses remain the same within the same type of variety, e.g. all red leaf or romaine lettuce react the same under the same spectral treatments (Ouzouniz, 2015; Samuoliene, 2011). Some LEDs on the market now have greater PAR efficacy than HID (Wallace and Both 2016), but more work is needed to test fixture biomass efficacy, to determine if their adoption will ultimately lead to reduced energy use by the controlled environment agriculture (CEA) industry.

The objective of this study was to first choose several varieties of lettuce for HPS and LED lighting comparisons after an initial qualitative evaluation with 25 cultivars. Our subsequent objectives were to determine the influence of LED and HPS lighting on the yield, morphology, and sensory properties of several cultivars of hydroponic lettuce. Finally we wanted to determine the biomass efficacy of each

lighting system. This portion of this thesis work contains the cumulative results of two years of lettuce variety trial work to achieve these objectives.

Methods and Materials

This project consisted of three separate experimental phases of growth and data collection activities.

Experiment 1, qualitative cultivar selection

Growth cycles took place in a glass greenhouse between February 2017 and December 2017 in Ithaca, NY (42° N latitude). During this phase, a 20 channel NFT system (FarmTek, South Windsor, CT) was constructed. The system consisted of 20 nutrient film technique (NFT) channels with alternating plant placement holes, there were 18 per channel with 8-inch spacing center to center. The 125-gallon reservoir was prepared with 100 gallons of nutrient solution prepared by combining Ultrasol (Allentown, PA) with Calcium nitrate to achieve a nutrient solution concentration of: 150 mg·L⁻¹ NO₃-N. The nutrient solution pH and EC were monitored daily and maintained between 5.5 to 6.5 and 1.9 and 2.1 dS·m⁻¹, respectively. The nutrient solution was prepared with tap water at the beginning of every new production cycle and replaced thereafter every two weeks. Water levels were topped off from municipal tap water once per week to every three days depending on plant age. Prior to transplanting in NFT channels, seeds were sown into 1" Oasis Horticubes XL (Oasis, Kent, OH) and grown under HPS lamps for approximately 21 days before being moved into the main treatment tables at the fourth leaf stage. An array of 16 high pressure sodium lights (PL Lighting, Beamsville, ON, Canada) was used, each with a 1000-watt,

single ended bulb. Photoperiod control was maintained through Argus, and set at a fixed period of 8am to 10pm every day during the summer and 6am to midnight during early spring and fall through winter months. Temperature set points were 21/17 °C day temperature/night temperature.

A water sample was taken from the tap supplied to the greenhouse range on September 14, 2017, and analyzed by the Cornell Nutrient Analysis Laboratory (Bradfield Hall, Ithaca, NY). Water was analyzed for Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, P, Pb, S, Si, Sr, V, and Zn. Relevant values are included in table 1. Elements that tested under .15 mg/L are omitted from the results analysis. The highest values detected are calcium, sodium, magnesium, and sulfur.

Table 1: Water analysis of greenhouse tap water, with values listed in mg/L (PPM).

Sample	Ca 211.276	K 766.491	Mg 279.079	Na 330.298	P 213.618	S 182.034	Si 251.612
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
9/14/2017	63.90	1.57	14.14	50.13	0.41	11.96	1.29

Solid nutrient salts were provided to the study by the company sponsors. The sponsors indicated that they would like to use the one part nutrient solution Ultrasol supplemented by Calcinite to achieved higher nitrogen and calcium values. Tab 2 compares the nutrient breakdown of Ultrasol to that of the modified Sonneveld solution typically used by Cornell CEA projects.

Table 2: Nutrient breakdown of two fertilizer solutions. The lettuce study used a combination of Ultrasol + Calnit, as provided by company sponsors.

Nutrient	Total PPM Ultrasol + CalNit	Total PPM Sonneveld
Nitrogen	154.580	150
Phosphorus	47.648	31
Potassium	189.995	132
Magnesium	50.630	24
Sulfur	87.623	NA
Boron	0.487	0.160
Copper	0.682	0.023
Iron	2.921	1
Manganese	0.974	0.250
Molybdenum	0.097	0.024
Zinc	0.389	0.130
Calcium	153.677	90

Twenty five varieties of lettuce were test-grown in our system during this period, with six field varieties from Dole (Thousand Oaks, CA) and nineteen from Rijk Zwaan (Table 1).

Quantitative data was not collected during this experiment as heads were used for customer sampling by the project sponsor. Instead, qualitative data was collected as an initial survey to identify varieties of interest for continuing study. These data consisted of incidence of tip burn and premature bolting under our greenhouse conditions. A total of 6 production cycles were conducted. The experiment was arranged as a randomized complete block design with two blocks of 5-8 replicates per cultivars per block per production cycle.

Experiment 2: Response of thirteen lettuce cultivars to LED and HPS light

Experiment 2 consisted of three crop cycles from January 2018 to May 2018 in the same glass house as above. From seeding to harvest, each harvest cycle was 55, 55, and 53 days, respectively. Typical lettuce production following the Cornell Lettuce Production Handbook produces lettuce plants around 35 days, but our seedling

production area differed significantly from those recommendations in terms of light availability and temperature controls. Plants were seeded into Oasis Horticubes and remained in seedling stage, growing under HPS lights, for ~21 days until they reached the fourth leaf stage. During propagation seedlings were watered daily or as needed with 150 mg·L⁻¹ N from 20N-2.2P-16.6 K (Jack's Professional LXTM Water Soluble Fertilizer 21-5-20 All-Purpose, J.R. Peter's Inc. Allentown, PA) with 30 mg·L⁻¹ Mg added from MgSO₄·7H₂O. Upon transplanting into the NFT system, the plants were grown out for a period of 33, 30, and 32 days for crop cycles 1, 2, and 3 respectively. During this phase, the FarmTek NFT system was split into two 10 channel tables and separated by about 15 feet to minimize light interference. Both tables shared the nutrient reservoir which was maintained as described in experiment 1.

One table with 10 NFT channels was placed under an array of six 1000-W HPS lights as described in experiment 1. The second table was placed under an array of six LED fixtures (Lumigrow Pro 650e, Emeryville, CA) which were arranged in an identical pattern and height to the HPS, with their intensity adjusted using SmartPAR software (Lumigrow, Emeryville, CA) to match that of the HPS array (180 μmol·m⁻²·s⁻¹). The LEDs were adjusted to a 20% blue, 80% red ratio of light using the SmartPAR software. A daily light integral (DLI) of 17 mol/day was chosen. The daily light integral (DLI) under each array was maintained at 17 mol·m⁻²·d⁻¹ using the Light and Shade System Implementation (LASSI) algorithm (Albright, 2000). A quantum sensor (LI-190R, LI-COR, Lincoln, Nebraska) to measure photosynthetic photon flux density (PPFD) was placed at the canopy level at a representative location under each array. The quantum sensors were connected to an Arduino/Raspberry Pi combined

microcontroller system which logged PPFD and sent values via a wireless internet signal to a separate Raspberry Pi which ran the LASSI algorithm and every half-hour communicated light and shade decisions to the connected to the greenhouse environmental control system (Argus, Canada). Table 1 contains a summary of the on/off hours per each lighting array per harvest cycle for Experiment 2.

Table 3: A summary of the average and standard deviation of number of hours each lighting array spent turned on during experiment two, broken down by harvest cycle.

On/Off Hours	HPS	LED
Cycle 1 Avg \pm SD	19.8 \pm 5.41	18.2 \pm 5.6
Cycle 2 Avg \pm SD	15.5 \pm 6.59	13.0 \pm 7.89
Cycle 3 Avg \pm SD	9.8 \pm 6.65	9.9 \pm 5.54

Thirteen varieties of lettuce were grown in experiment two in each of the three cycles. The thirteen varieties were: ‘Rex’, ‘Teodore’, ‘Locarno’, ‘Xandra’, ‘Rouxai’, ‘Big Star’ (referred to as ‘Greenstar’), ‘Rocky Row’, ‘Carmessi’, ‘Crunchita’, ‘Lotus’, ‘Seurat’, ‘Aquino’, and ‘Barlach’. The experiment was arranged as a randomized complete block design with two blocks of 5-6 replicates per cultivars per block per lighting treatment with a total of 3 production cycles. Due to their large size, the romaine varieties were spaced to contain one empty plant slot between each head. For all three crop cycles data was collected on lettuce head fresh weight (with head separated from roots just above the NFT channel). In the first replicate, a taste test was conducted as described below. In the second replicate, Brix data was collected. In the third replicate, data were also collected on plant height (from the severed base to the highest part of the plant) and width (diameter of the widest part of the plant).

Sensory evaluation – The sensory evaluation was conducted the day after harvest using plants from the first crop cycle with the participation of 24 students and 1 professor as tasters. Evaluators were given a brief explanation of taste and texture responses, a bottle of water, and crackers as palate cleansers. One representative lettuce head of each variety, from each treatment (LED and HPS) was selected and presented to tasters in a single blind test, labelled as either A or B. The 13 stations were set up in a classroom and tasters moved from station to station, sampling a piece of leaf from each head and recording their responses on a worksheet. Three categories were evaluated: color, taste, and texture. The color category evaluated differences between green and red lettuce, and degree of redness between the LED and HPS treatments. The taste category labelled plants as sweet (3), mild (2), or bitter (1), with tasters asked to select one response. The texture category labelled plants as crisp (1), crunchy (2), or soft (3), with tasters asked to select one response. Responses were analyzed through the use of Chi squared tables.

Brix and glucose were recorded in crop cycle two in an attempt to collect some quantitative data which may be associated with flavor. We developed a protocol whereby 2 to 4 recently mature leaves from each lettuce head were placed in a quart-size freezer bag and placed in a freezer (-18 °C) for one week. Once frozen, samples were removed from the freezer, crushed and ground, and allowed to defrost. Juice was allowed to collect in the bags before being read with a handheld refractometer (SIM Supply, Hibbing, MN). Glucose measurements from 159 samples per cultivar were also taken using a One Touch (LifeScan, Inc., Milpitas, CA) blood glucose monitor to measure the same liquid samples used for Brix measurement. The range of the meter

used was 0 to 600 mg·L⁻¹. Brix testing was specifically requested by the company sponsors, while glucose was not.

Electrical Efficacy – To estimate the biomass efficacy of each lighting treatment (g edible fresh weight / kWh electricity) we summed the harvestable fresh weight from each treatment and then divided this by estimated electricity consumption. Electricity consumption was estimated by using logged data on the number of hours lighting arrays were on for each crop cycle and multiplying this by the instantaneous electricity use of each array (W). The instantaneous use of each array was estimated by plugging select fixtures into an electricity usage monitor. There are several qualifying statements that need to be made about biomass efficacy estimates. First, it is merely an estimate, as every light was *not* measured individually to check for electrical usage. Secondly, the HPS bulbs had already been used for several thousand hours while the LED fixtures were new. Third, no effort was made to quantify the amount of unused light lost to the perimeters of the growing area. Nevertheless, we feel studies with food crops and lighting should report biomass efficacy as observed under their experimental conditions.

Experiment 3: performance of select lettuce cultivars in response to HPS and LED light

Due to fairly large plant to plant variability noted in experiment 2, we decided to conduct three additional growth cycles with five select cultivars, to allow for more replicates per cultivar per lighting treatment. The three crop cycles took place during June to October 2018, and the crop cycle length was 55, 55, and 53, days for crop cycles 1, 2, and 3, respectively.

Statistical analysis were performed in R (RStudio, Version 1.1.414, the R Foundation) by employing ANOVA tables and mixed effect linear models. The experiment was set up in a randomized block design through experiments II and III, with random effects of harvest cycle and block taken into consideration. Fixed effects of light and variety, and interactions between light and variety, were factored in (Appendix II). After controlling for fixed effects and their interaction, a mean separation comparison was conducted to compare cultivars using a post hoc Tukey's correction. Taste test results were analyzed using χ^2 test of independence tables. Responses were summed per category and χ^2 values were calculated and p-values obtained for each set of factors, separated by variety. For Texture and Taste, degrees of freedom were 2; for color, degrees of freedom were 3. Sample size was between 44 and 50 for each variety in each factor, as some respondents elected not to sample certain varieties. Expected values were chosen based on the null hypothesis that there was no difference in consumer perception (total number of responses for one category[0-50] / number of treatments [2]).

Results

Experiment 1

A set of selection criterion were developed during experiment 1 for choosing the most appropriate varieties moving forward. Lettuce varieties were separated into several categories: green romaine, red leaf lettuce, green butterhead, green large leaf lettuce, and green small leaf lettuce. There were no true red head or red romaine lettuce varieties. Two or more of the most promising varieties within each category were selected for subsequent experiments.

Table 4. Qualitative assessment of 25 lettuce varieties in experiment 1 for tip burn, bolting, and size. TB indicates presence of tip burn at harvest; B indicates bolting at harvest and x indicates not observed.

Variety	Type	Color	Tip Burn	Bolting	Size	Provider
Carmessi	Leaf	Red	x	x	Medium	Rijk Zwaan
Chicarita	Romaine	Green	TB	x	Small	Rijk Zwaan
Rex	Butterhead	Green	x	x	Medium	Rijk Zwaan
Barlach	Leaf	Red	x	x	Medium	Rijk Zwaan
Flandria	Butterhead	Green	x	x	Small	Rijk Zwaan
Xandra	Leaf	Red	x	x	Medium	Rijk Zwaan
Livorno	Leaf	Green	TB	B	Small	Rijk Zwaan
Rafael	Romaine	Green	TB	B	Small	Rijk Zwaan
Cosmopolita	Head	Green	TB	B	Small	Rijk Zwaan
Rouxai	Leaf	Red	x	x	Medium	Rijk Zwaan
Lotus	Romaine	Green	TB	x	Small	Rijk Zwaan
Pascal	Leaf	Green	x	B	Small	Rijk Zwaan
Teodore	Leaf	Red	x	B	Large	Rijk Zwaan
Seurat	Leaf	Red	TB	x	Medium	Rijk Zwaan
Aquino	Butterhead	Green	TB	B	Small	Rijk Zwaan
Locarno	Leaf	Green	TB	x	Med/Small	Rijk Zwaan
Rocky Row	Romaine	Green	TB	x	Large	Dole
Sun Valley	Romaine	Green	TB	x	Large	Dole
Greenstar	Leaf	Green	x	x	Large	Dole
Sunbelt	Romaine	Green	TB	x	Large	Dole
Fort Romie	Romaine	Green	TB	x	Large	Dole
Solid King	Romaine	Green	TB	x	Large	Dole
Crunchita	Romaine	Green	TB	x	Small	Rijk Zwaan
Verodita	Leaf	Green	x	x	Large	Rijk Zwaan
Corentine	Head	Green	x	x	Medium	Rijk Zwaan

Tip burn

In a greenhouse, tip burn issues can be caused by a variety of environmental conditions. These can include over-lighting, under-ventilation leading to high humidity,

and lack of calcium in the nutrient solution. During summer months, plants in experiment 1 suffered from over lighting (due to use of time clock-based lighting control) and high humidity conditions, which lead us to identify varieties that exhibited early tip burn. Romaine varieties exhibited more server tip burn than other categories. The Romaine cultivars also tended to be the highest yielding category. Larger heads of lettuce may have a rate of growth that outpaces the speed at which calcium can traverse through the plant, more readily allowing for tip burn to occur. Our other large variety, Greenstar, was a loose-leaf variety whose growing point was less enclosed than the Romaine varieties, which may have allowed for a greater rate of transpiration around the growing tip and helped to combat tip burn. Greenstar rarely exhibited symptoms of tip burn.

Bolting

Since bolting can be triggered by several different environmental factors including high air temperature and long day photoperiod (Simone et al, 2002; Nothmann, 1977), it is important to select varieties that have compatible bolting tendencies in order to synchronize the timing of harvest. Experiment 1 (which had lights controlled by a time clock rather than according to daily light integral targets) exhibited the most bolting issues, and several varieties were identified and eliminated based on their tendency to bolt earlier than the majority of other varieties (Table 1). Bolting was a much less common response in experiment 2, however, Theodore and Pascal consistently began bolting up to a week before harvest in almost all harvest cycles. Theodore was identified by the seed supplier as a lettuce variety suitable for a

photoperiod of 13 hours or less, suggesting the long photoperiod of our lighting treatments was involved in bolting.

Size characteristics

Size at harvest for lettuce varieties is determined by cultivar, environmental conditions, and crop time. In our variety screen, we wanted to identify consistently larger varieties (as sales price is dependent on fresh weight). We focused on head lettuce rather than leaf lettuce. Some field varieties Rocky Row, Greenstar) were also included in order to compare their performance to varieties not bred specifically for field production. Most small varieties were ultimately not used in subsequent experiments.

Experiment 2

Fresh Weight

For twelve of the thirteen varieties, lighting treatment did not significantly impact fresh weight. In the case of Theodore plants under LED were smaller than HPS. However, it should be noted that Theodore heads were almost always bolting at harvest and this was more prominent under HPS.

In comparing the fresh weight of varieties to each other mean fresh weight varied from 53.42 to 227.7 g. The largest varieties produced were Greenstar (HPS: 189.79 ± 20.99 g) and Rocky Row (HPS: 227.70 ± 20.8 g), both of which were field varieties provided by Dole. The smallest varieties were Locarno (HPS: 53.42 ± 20.83 g) and Carmessi (57.33 ± 20.83 g). The consistency of plants within a variety is indicated by the standard error. While Greenstar and Rocky Row were our largest varieties, they also had the widest range of possible final harvest sizes. This variability could make difficult

to reach a consistent harvest size by a given date. Our varieties with the least variability across harvests were Carmessi and Rex, though many of the smaller varieties were fairly consistent producers.

Table 5. Fresh weight (FW) of thirteen cultivars of lettuce in experiment 2 in which plants were grown under HPS or LED lights with a target DLI of $17 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. Data are means \pm standard error (SE) of ca. 36 plants from three crop cycles, with Tukey's grouping broken down by lighting treatment and variety. A Tukey's HSD was performed to further clarify relationships between HPS and LED varieties.

Variety	HPS FW (g)	LED FW (g)	P Value	Tukey's HPS	Tukey LED
Aquino	71.24 \pm 20.83	66.34 \pm 20.85	0.677	ABC	AB
Barlach	102.83 \pm 20.78	102.97 \pm 20.78	0.990	CBCDEF	BCDEF
Carmessi	57.33 \pm 20.83	64.41 \pm 20.78	0.544	A	AB
Crunchita	132.97 \pm 20.78	143 \pm 20.87	0.393	DEFG	FGH
Greenstar	189.79 \pm 20.99	183.81 \pm 20.83	0.619	IJK	HIJ
Locarno	53.42 \pm 20.83	62.69 \pm 20.83	0.430	A	AB
Lotus	166.48 \pm 20.78	164.95 \pm 20.76	0.895	GHI	GHI
Rex	111.35 \pm 20.76	111.07 \pm 20.8	0.981	CDEF	CDEF
RockyRow	227.7 \pm 20.8	225.59 \pm 20.8	0.856	K	JK
Rouxai	70.1 \pm 20.8	70.71 \pm 20.81	0.958	ABC	ABC
Seurat	94.09 \pm 21	90.29 \pm 20.8	0.751	ABCDE	ABCD
Teodore	171.13 \pm 22.16	140.11 \pm 21.71	0.042	GHI	EFGHI
Xandra	93.37 \pm 20.76	86.08 \pm 20.8	0.529	ABCDE	ABC

Fresh weight response to lettuce category

The thirteen lettuce varieties were separated into four categories: red (Carmessi, Xandra, Rouxai, Barlach, Seurat, and Teodore), green (Locarno and Greenstar), butterhead (Rex and Aquino), or Romaine (Rocky Row, Lotus, and Crunchita), to see if any fresh weight patterns emerged when viewed by category. No significant differences between HPS and LED weights were observed when data were analyzed according to these categories.

Category	HPS	LED	P Value
Butterhead	91.65	88.68	0.865
Green	116.6	123.4	0.703
Red	92.98	89.27	0.724
Romaine	175.71	178.31	0.856

Table 6. Fresh weight of all lettuce heads when separated by categories instead of variety. Data are means of the two lighting treatments (HPS vs LED). P values indicate significance of difference in means.

Taste Test

Using χ^2 test of independence, several varieties showed significant differences in both color and taste perception. Regarding taste, Teodore was perceptibly more bitter under HPS with 17 responses indicating bitter, 7 mild, and 1 sweet, while LED plants trended more toward mild with 12 responses, 8 responses indicating bitter, and 5 indicating sweet. The χ^2 valued for this table was 0.027. Seurat and Rouxai had nearly significant differences in flavor, with p values of 0.105 and 0.113, respectively. Regarding color, Carmessi, Rouxai, and Seurat all had significant differences in color perception, with all varieties being perceptibly more red under LED than HPS. Interestingly, our other red varieties showed no perceptible differences in color between HPS and LED according to consumer responses. There were no significant differences in texture between any varieties, though this could have been due to poor understanding of texture categories. Respondents struggled the most during the explanation stage of the texture category.

Table 7: Three χ^2 tables of lettuce raw response data. Only varieties and factors that were found to be significant are shown. Two tables of color responses and one table of taste responses showed significant χ^2 values.

Rouxai Color					
	Red	Mixed	Dark Gree	Light Green	Total
HPS	0	22	0	0	22
LED	10	12	0	0	22
Total	10	34	0	0	44

Seurat Color					
	Red	Mixed	Dark Gree	Light Green	Total
HPS	0	20	5	0	25
LED	10	14	1	0	25
Total	10	34	6	0	50

Teodore Taste				
	Bitter	Mild	Sweet	Total
HPS	17	7	1	25
LED	8	12	5	25
Total	25	19	6	50

Brix

Brix and glucose values were taken in crop cycle 1. Table 6 lists the mean and p value measured for each cultivar (sample loss and inconsistency of glucose test strips meant that not every plant was measured for Brix and glucose).

Brix values averaged between 2 to 5. Greenstar and Rouxai were the only two varieties that had average brix values higher under HPS; all other varieties averaged higher Brix under LED (Table 6). The variety with the highest average brix was Greenstar, with an average of 5.19 brix, while the lowest was Barlach, with an average brix of 2.74.

A statistical model for brix results was designed incorporating Lights, Variety, position within tables, and the interaction factor between Variety and Lights. No

statistically significant differences were found to an alpha of 0.1, although this may be attributed to a small sample size being used. As seen with height and width data, significant differences were found once sample size was increased from 60 total to 216 total per variety. Brix data collection was only performed once, for a max sample size of 20 total per each variety, with half of the samples being under each treatment. Some varieties approached an alpha of 0.1, but none were less than 0.14.

A Tukey's multiple comparison test was performed to identify which varieties under which treatments were significantly different from each other or could be grouped together. Five separate groups were identified, though most varieties and treatments were included in three or more groups.

Table 8: Brix means \pm SE broken down by variety and lights. P value indicates the significance in difference between means. A Tukey's grouping was performed for further clarify differences.

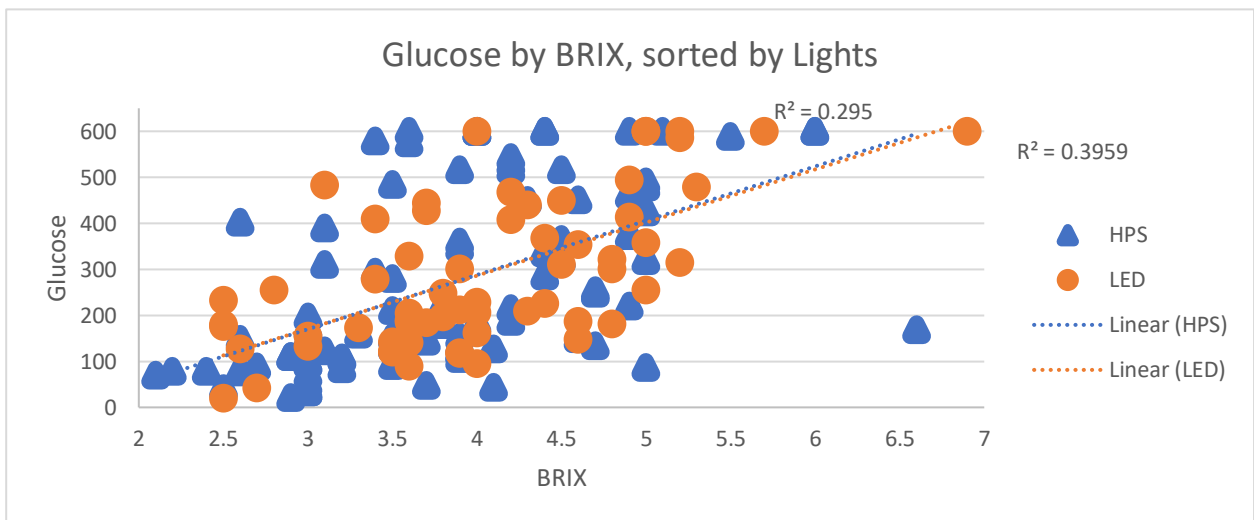
Variety	HPS Brix	LED Brix	P Value	Tukey's HPS	Tukey's LED
Aquino	3.02 \pm 0.29	3.65 \pm 0.3	0.149	AB	ABCDE
Barlach	2.74 \pm 0.29	3.17 \pm 0.29	0.310	A	ABC
Carmessi	4.25 \pm 0.28	4.85 \pm 0.29	0.146	ABCDE	DE
Crunchita	4.79 \pm 0.29	4.78 \pm 0.31	0.985	CDE	CDE
Greenstar	5.16 \pm 0.29	4.62 \pm 0.29	0.202	E	BCDE
Locarno	4.43 \pm 0.29	4.91 \pm 0.28	0.236	ABCDE	DE
Lotus	3.8 \pm 0.29	3.87 \pm 0.29	0.864	ABCDE	ABCDE
Rex	3.42 \pm 0.29	3.48 \pm 0.29	0.883	ABCD	ABCDE
RockyRow	4.31 \pm 0.29	4.93 \pm 0.29	0.143	ABCDE	DE
Rouxai	3.44 \pm 0.29	2.88 \pm 0.29	0.187	ABCD	A
Seurat	3.43 \pm 0.29	3.52 \pm 0.27	0.827	ABCD	ABCD
Teodore	3.48 \pm 0.35	4.2 \pm 0.28	0.116	ABCDE	ABCDE
Xandra	3.48 \pm 0.29	3.95 \pm 0.29	0.257	ABCDE	ABCDE

Glucose

Because personal glucose monitors are available inexpensively in the medical market, we wished to determine if lettuce glucose (as measured using test strips) correlated with brix. There was a slight positive correlation between glucose and brix with an R^2 of 0.295 for HPS and 0.396 for LED (Figure 1).

A statistical model for glucose results was designed to look at the relationship between brix and glucose, including the factors of Lights, Variety, position within tables, and the interaction between Lights and Variety. No significant differences were detected to an alpha of 0.1, but sample size was not consistent among varieties or treatments. Again, only one harvest cycle was used to record measurements, for a max sample size of 20 total. Due to equipment failures and sample loss, some varieties under individual treatments had as few as 2 samples, and up to as many as 10.

Figure 1: Recorded glucose responses graphed against recorded brix values. Data are raw responses of individual plants that were read for both brix and glucose. Orange dots represent LED-grown plants, and blue triangles represent HPS-grown plants. Regression trend-lines are overlaid.



To analyze the relationship between glucose and brix values, an ANOVA analysis was run on the glucose model, which analyzed the significance of brix, variety,

light, brix:light, and variety:light. Two terms were found to be significant predictors of glucose values: brix, and variety. These results indicate that observed brix values most likely correlate strongly to glucose (sugar) results, and that the results are strongly variety dependent. Importantly, light treatment did not appear to affect results. Given the previously discussed data issues, however, I would avoid drawing any strong conclusions without gathering more data to increase sample size. Future work in this area could be done using better equipment with a larger number of plants.

Table 9: ANOVA table of glucose model. Two significant factors in the glucose model are identified: brix and variety. Lighting treatment does not have a significant impact on glucose results.

	NumDF	F value	P Value	
BRIX	1	32.9266	6.73E-08	***
Variety	12	5.9206	4.24E-08	***
Light	1	0.0813	0.776	
Variety:Light	12	0.5843	0.8516	
BRIX:Light	1	0.1496	0.6995	

Height

Data was collected on height for the third crop cycle. At an alpha of 0.05, significant differences between HPS and LED lettuces were noted in two varieties: Lotus and Rocky Row. In both cases HPS plants were taller than LED. Both varieties belong to the Romaines group and are two of the tallest varieties grown. At an alpha of 0.1, Greenstar, was also taller under HPS than LED.

Table 10: Height means in centimeters of plants in experiment 2, cycle 3. Data are means \pm SE of HPS and LED plants, with p values representing the significance in difference of means. A Tukey's HSD was performed to further clarify relationships between varieties and treatments.

Variety	HPS Height (cm)	LED Height (cm)	P Value	Tukey's HPS	Tukey's LED
Aquino	11.45 \pm 0.73	11.18 \pm 0.73	0.793	AB	A
Barlach	14.5 \pm 0.73	13.68 \pm 0.73	0.432	ABCD	ABC
Carmessi	16.73 \pm 0.73	15.86 \pm 0.73	0.407	CDE	CDE
Crunchita	18.8 \pm 1.09	18.59 \pm 0.73	0.874	DEF	E
Greenstar	25.95 \pm 0.73	24.18 \pm 0.73	0.089	GHI	GHI
Locarno	13.75 \pm 0.77	15.23 \pm 0.73	0.167	ABC	BCDE
Lotus	26 \pm 0.73	22.82 \pm 0.73	0.002	GHI	FG
Rex	13.14 \pm 0.73	13.09 \pm 0.73	0.965	ABC	ABC
RockyRow	29.32 \pm 0.73	27.09 \pm 0.73	0.033	I	HI
Rouxai	17.77 \pm 0.73	17.68 \pm 0.73	0.930	DE	DE
Seurat	14.73 \pm 0.73	14.68 \pm 0.73	0.965	ABCD	ABCD
Xandra	16.14 \pm 0.73	15.09 \pm 0.73	0.315	CDE	BCDE

Diameter

Data on plant diameter data (width at widest point) were also collected from the third crop cycle in experiment 2. At an alpha of 0.05, there were no statistically significant differences observed within our data plants. At an alpha of 0.1, only one variety, Rouxai, had a significantly different diameter in response to light treatment

Table 11: Diameter in centimeters of plants in experiment 2, cycle 3. Data are means in cm \pm SE of HPS and LED plants, with p values representing the significance in difference of means. A Tukey's HSD was performed to further clarify relationships between varieties and treatments.

Variety	HPS Width (cm)	LED Width (cm)	P Value	Tukey's HPS	Tukey's LED
Aquino	18.64 \pm 1.88	17.73 \pm 1.88	0.737	A	A
Barlach	23.22 \pm 1.88	21.98 \pm 1.88	0.645	ABCD	ABC
Carmessi	24.99 \pm 1.88	23.65 \pm 1.88	0.621	ABCD	ABCD
Crunchita	29.4 \pm 2.71	26.06 \pm 1.88	0.321	ABCD	ABCD
Greenstar	31.67 \pm 1.88	31.66 \pm 1.88	0.998	BCD	BCD
Locarno	19.46 \pm 1.92	20.85 \pm 1.88	0.611	A	AB
Lotus	28.01 \pm 1.88	27.98 \pm 1.88	0.992	ABCD	ABCD
Rex	21.37 \pm 1.88	20.34 \pm 1.88	0.703	ABC	A
RockyRow	32.27 \pm 1.88	34.12 \pm 1.88	0.494	CD	D
Rouxai	24.64 \pm 1.88	20.56 \pm 1.88	0.138	ABCD	A
Seurat	20.45 \pm 1.88	20.52 \pm 1.88	0.980	A	A
Xandra	27.66 \pm 1.88	24.53 \pm 1.88	0.252	ABCD	ABCD

Biomass Efficacy

Edible mass is defined as the total head weight of lettuce. Electricity consumption was greater under HPS than LED (Table 9). For harvest 1, HPS lights produced a total of 7.67 g edible mass per estimated kWh electricity, while LEDs produced a total of 18.57 g. For harvest 2, HPS produced 13.83 g, while LED produced 42.42 g. For Harvest 3, HPS produced 25.70 g, while LED produced 66.04 g. Therefore, under the conditions of our experiment, the LED treatment led to a 2.4 to 3.1 times biomass efficacy than HPS. As noted in the materials and methods, several cautions must be taken with these estimates, such as age of lights, electrical use variations, and wasted light.

Table 12: Electrical use and biomass efficacy of harvests 1-3 in experiment 2. Total kilowatt hours (kWh) used and total edible biomass produced in grams were used to calculate biomass efficacy, a measure of edible mass produced per unit energy consumed.

Harvest 1		Harvest 2		Harvest 3		
HPS	LED	HPS	LED	HPS	LED	
2400	948	2400	948	2400	948	Watts per Array
1618	586	1119	369	754	301	Total kW hr Used
47	17	37	12	23	9	Average Daily kW hr
12411	10888	15475	15678	19393	19921	g Edible Mass Produced
7.67	18.57	13.83	42.42	25.70	66.04	g Edible Mass / kW hr

Experiment 3

Weight

In experiment three, we grew only six varieties of lettuce to increase our sample size per variety. Even at an alpha of up to 0.1. there were no significant differences found in fresh weight between HPS and LED treatments.

Table 13: Weight means of six varieties in experiment 3. P values indicate the significance in difference in means between HPS and LED treatments. Tukey's HSD was omitted as all varieties and treatments fell within the same group.

Variety	HPS FW (g)	LED FW (g)	P Value
Crunchita	109.11 ± 14.68	115.13 ± 14.68	0.397
Greenstar	152.29 ± 14.68	151.42 ± 14.68	0.902
Locarno	59.16 ± 14.7	61.95 ± 14.71	0.699
Rex	101.05 ± 14.68	108.88 ± 14.68	0.272
Rouxai	103.03 ± 16.62	101.08 ± 16.46	0.875
Xandra	73.74 ± 15.14	68.46 ± 15.14	0.542

Height

Significant differences were detected in both Greenstar and Xandra (Table 11).

HPS Greenstar diameter mean was 1.87 cm larger than LED (13.66 +/- 0.4 HPS vs 11.79 +/- 0.66 LED), while LED Xandra was 3.72 cm larger than HPS Xandra (15.65 +/- 0.48 LED vs 11.93 +/- 0.66). All other varieties had no significant differences between groups. Both fresh weight and height of Locarno were highly variable, suggesting it is not a reliable variety under the environmental conditions of our experiment.

Table 14: Height differences in centimeters of plants in experiment 3. Data are means ± standard error, with p values indicating significance in difference of means.

Variety	HPS Height (cm)	LED Height (cm)	P Value	Tukey's HPS	Tukey's LED
Crunchita	18.59 ± 0.4	22.36 ± 0.41	0.567	A	A
Greenstar	13.66 ± 0.4	11.79 ± 0.66	0.010	B	A
Locarno	18.25 ± 0.4	14.8 ± 0.41	0.366	A	A
Rex	13.45 ± 0.41	17.08 ± 0.48	0.726	A	A
Rouxai	24.19 ± 0.41	14.25 ± 0.41	0.882	A	A
Xandra	11.93 ± 0.66	15.65 ± 0.48	0.047	B	A

Diameter

Crunchita, Greenstar, Locarno, and Xandra exhibited significant differences in means between HPS-grown and LED-grown plants (table 12). HPS Crunchita plants were 1.2 cm larger than LED plants. HPS Greenstar plants were 1.8 cm larger than LED plants. HPS Locarno plants were 1.21 cm larger than LED plants. HPS Xandra plants were 1.77 cm larger than LED plants.

Table 15: Diameter in centimeter of plants in experiment 3. Data are means \pm standard error, with p values included to show significance of differences in means between treatments. Tukey's HSD was included to further clarify relationships between varieties and treatments.

Variety	HPS Width (cm)	LED Width (cm)	p Value	Tukey's HPS	Tukey's LED
Crunchita	24.91 \pm 0.4	23.71 \pm 0.4	0.035	B	A
Greenstar	28.09 \pm 0.41	26.24 \pm 0.4	0.001	B	A
Locarno	17.9 \pm 0.4	16.69 \pm 0.41	0.037	B	A
Rex	19.21 \pm 0.4	18.46 \pm 0.4	0.192	A	A
Rouxai	22.33 \pm 0.69	21.4 \pm 0.69	0.342	A	A
Xandra	22.81 \pm 0.49	21.04 \pm 0.49	0.011	B	A

Electrical Efficacy

We were not able to calculate electrical efficacy in experiment 3, as the data tables with fixture on/off times was corrupted.

Discussion

Growing lettuce in greenhouses often requires supplemental lighting during offseason production as ambient light levels drop, depending on geographical location of greenhouses. As such, the debate over what type of supplemental lighting to use continues to evolve as technology rapidly changes. Interestingly in 2014 the electrical efficacy of evaluated LED fixtures was no better than the best HPS fixtures (Nelson and Bugbee). However, by 2016 the best evaluated LED fixture were 40% more efficacious than HPS ((Wallace and Both, 2016). Historically HID fixtures have been used for supplemental lighting, but LED adoption is occurring with an estimated 2% of U.S. lit greenhouse area using LEDs (Stober et al, 2017). A difficulty with LED is the high initial capital investment. In 2014 LED fixtures tended to cost five to ten times more than HPS (Nelson and Bugbee, 2014). Previous studies have also observed significant energy saving when using HPS over LED lights in the production of lettuce (Martineau, et al, 2012).

The results of this study indicate that similar growth and appearance is achievable through the use of LEDs, which comes with a significant savings in electrical operations cost. Our study found few yield or morphological effects of light source. Martineau, et al., found a similar result with Boston lettuce, but there was no inclusion of red leaf varieties (2012). This study also combined ambient sunlight with supplemental HPS and LED fixtures, using red and blue LEDs comparable to those used in the current study. Another study published in 2017 compared the differences in growth of one type of green head lettuce grown in the field under different colored shade clothes and did find significant differences between red and blue colors when comparing head weights and diameters (Ilic et al., 2017). Lettuce diameter is affected by leaf expansion, and the 2017 study observed greater leaf area and larger diameters under red shade clothes. In the current study only once sample size was increased two fold did difference begin to become significant: taller and wider heads were observed in several varieties under HPS lighting as opposed to the LED treatment, which contained a higher proportion of blue light. Though these results were significant, the practical significance with differences of 1-2 cm can be argued.

The relatively minor impact of light treatment in our experiment and those previous may also be because our experiment as in a greenhouse with a background of sunlight. The spectral differences in HPS vs. LED fixtures appear to have minimized by ambient sunlight as was reported by (Li, 2009). Consumer perception of taste and texture tended to be fairly similar, with a two varieties reported to be slightly more bitter under HPS and red leaf varieties were more red under LED treatments (Stutte, 2008; Simonne, et al, 2002). Anthocyanin production in response to blue light is a well

quantified response in certain lettuce varieties. Stutte et al (2009), working with red leaf lettuce, found that wavelength of light provided in a sole source lighting environment had a dramatic effect on anthocyanin production as well as total plant growth when compared to fluorescent lighting of the same intensity. A red-blue spectrum produced four times as much anthocyanin content as a red spectrum alone, when grown at the same intensities. However, these are large differences, but the work was conducted in growth chambers, under sole-source lighting. In our experiment, the sensory panel, differences in red pigmentation of red leaf lettuce were noticed, however differences were relatively subtle which may be due to the presence of sunlight or the degree of blue light used in our LED treatments (20%).

HPS and LED lights have a significant difference in distribution patterns that can make the choice between the two of them very application dependent. LEDs tend to have a narrower focus in lighting area, better lending them to use in greenhouses that have aisles, benches, or walk ways. HPS lights have a broader distribution, enabling them to better cover wider areas more evenly (Nelson and Bugbee, 2014). These distribution pattern differences will lead to a difference in hanging distribution of lights and potentially affect the number of lights a grower will need for even coverage. The present study has shown that LEDs can offer a significant savings in electrical consumption while producing the same quality of lettuce, but the grower must still decide if the start-up costs are worth the savings over time. a thorough study of lights required should be conducted for each operation individually.

The current study had several weaknesses that should be dealt with in any further work conducted. The location of LED and HPS treatments in the greenhouse was not

rotated between experiment because of the difficult accessing fixtures above NFT tables and remounting. In terms of light the location effect was mitigated by using quantum sensors to control lighting treatments. A second issue was the current implementation of the LASSI algorithm used to control lighting treatments in the second and third experiments. Primarily due to wireless connectivity issues the algorithm was not able to always achieve the DLI target for each treatment.

In conclusion, variety selection for hydroponic lettuce production within a greenhouse must take several factors into account, but lighting may have less of an impact than some growers are aware of. Large savings in electrical consumption can be achieved through light selection, control, and distribution, while still producing lettuce of consistent size and appearance.

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CHAPTER 2

RESPONSE OF TOMATO GROWTH, YIELD, AND ASCORBIC ACID CONTENT TO VARYING RATIOS OF RED AND BLUE SOLE-SOURCE LIGHTS

Abstract

Tomatoes are one of the most widely grown fruiting crops in the world. There is interest in manipulating environmental growing conditions to increase nutritional content. For example, previous researchers found that use of ultraviolet (UV) light could enhance ascorbic acid content and improve plant development timelines. However, the use of UV light and light emitting diodes (LEDs) is somewhat problematic: UV LEDs currently are more expensive than other types of LEDs, and UV light itself can be harmful to both crops and workers if not properly and rigidly controlled. If effective, the use of blue light may represent a reasonable alternative to UV as it is both cheaper and safer for both plants and workers. One objective of this experiment was to determine if blue light could be used to enhance ascorbic acid content, without negatively impacting plant quality and yield. In this experiment, three red:blue ratios (90:10, 70:30, and 55:45) of sole source LED lighting were investigated with one broad spectrum white light treatment as a control. The plant material used was tomato, *Solanum lycopersicum* L., cultivar ‘Micro Tom’, a dwarf cultivar which is quick to fruit. Data was collected on seedling height, flower and fruit production rates, fruit number, fruit weight, fruit size, Brix in fruit, and ascorbic acid contents per treatment.

90:10 and 55:45 treatments showed a significant effect on seedling height. Significant differences in number of fruit produced were observed, with 90:10 producing the most and 70:30 producing the least. Number of developing fruits and flowers were both affected by treatments, with significant differences observed between most treatments over time. Ascorbic acid showed no significant differences between treatments. There were no significant differences observed between treatments in harvest weight, fruit size, or brix.

Introduction

Micro Toms are a dwarf variety of tomato that stay compact (10-20 cm in height) and have a life cycle of 70-90 days from seed to mature fruit (Flores et al, 2016). Fruit yield and vitamin C content of Micro-Tom fruit was not impaired in response to macronutrient deficiency, demonstrating this variety's tolerance to environmental stresses (Flores et al, 2016). Beyond their morphological and nutritional benefits, Micro Tom is a useful model system for molecular work due to the availability of a reference genome, recent transcriptomic work, and with fruit producing transgenic plants able to be generated in as short as four months (Cruz-Mendivil, et al., 2011).

Vertical farms popular in urban agriculture and bioregenerative life support systems (BLSS) developed by NASA are similar in that both have significant limitations in both space and resources. Vertical farming prioritizes utilization of land not otherwise used for food production in urban environments to deal with issues such as food security, carbon footprint, and public education (Association of Vertical Farming). Many vertical farming setups select small yet productive crops. BLSS combine

bioregenerative processes utilized by plant growth and production to recycle resources through production systems. Plants are selected both by nutritional value and horticultural characteristics (Mitchell, 1994). Given their small size, Micro Tom may be a suitable plant for vertical farms (multi-layer indoor production) and plant density can be increased to optimize the limited growing area available in a space flight environment (Massa, 2016).

Vertical farming typically relies on sole-source lighting to drive photosynthesis. The response of tomato plants to sole source lighting (i.e. electrically derived light in the absence of sunlight) has not yet been thoroughly investigated. Several factors have played a role in this dearth of research. In general, the growth habit (tall plant height) of tomatoes does not lend itself well to controlled growth in a growth chamber; tomatoes are typically grown in greenhouses. LED technology has evolved rapidly in recent years, allowing greater specificity of spectral quality to be achieved and enabling researchers to observe the effects of more narrow wavebands (Massa, 2008). Some research that is available has focused mostly on augmentation of nutritional content of the tomato fruits themselves, rather than whole plant morphology. A response in tomato fruits to UV treatments pre- and post-harvest was observed in several recent studies (Castagna et al, 2014 ; Dzakovich et al, 2016). An increase of antioxidant activity and polyphenol content in both the peels and the flesh of tomato fruits was observed after treatment with UV-B radiation for just one hour a day (Castagna et al, 2014). Dzakovich, Ferruzzi, and Mitchel (2016) grew greenhouse tomatoes under supplemental, environmentally relevant doses of UV-B radiation to measure response in antioxidant production but

found no significant changes. However, supplemental UV-A lighting did produce a change in consumer perception of acidity and aroma.

Plants are able to respond to light quality through the presence and activity of several different classes of photoreceptors. Each class is sensitive to a different band of light. Phytochromes are sensitive to red and far red light; cryptochromes are sensitive to blue light and portions of the UV range; phototropins and zeitlupes are further sensitive to light in the blue range (Pocock, 2015). Specific morphological responses are associated with each photoreceptor and have been well documented in various crops. The manipulation of red to far red ratio to impact phytochrome photoequilibria can influence seed germination, shade avoidance behaviors, flowering, and changes in plant height (Pocock, 2015).

The photoreceptor most directly involved in reaction to the presence of blue light is the cryptochrome. Cryptochromes are involved in the production of anthocyanins, more compact plant size, and stomatal opening (Pocock, 2015). Phototropins appear to have more fast-acting influences, such as control of leaf movement and stomatal opening (Pocock, 2015). Gilberto, et al (2005) investigated the role of cryptochrome 2 (cry2) in tomatoes and found that this photoreceptor was involved in shortening of hypocotyls in seedlings and shortening of internodes in adult plants under blue light, earlier flowering under short days, and involvement in antioxidant production within fruit when overexpressed in mutants. These results indicate that changes in spectral environment produce relevant changes as they fall upon relevant receptors both within vegetative and fruiting organs.

Light intensity and quality dramatically affect the morphology and nutrient content of many food crops (e.g. Zushi et al, 2014; Kopsell et al., 2015; Lopez et al, 2016). Red leaf lettuce demonstrates an increased pigmentation response due to anthocyanin accumulation when grown under high levels of blue light (Stutte et al, 2009), as well as accumulating more biomass when grown under primarily red light (Heo et al, 2012). Kopsell (2015) investigated the effects of blue wavelengths on a variety of crops and found that parameters ranging from pigment accumulation to increases in primary or secondary metabolite production were affected in brassicas and microgreens. Some of these effects have been posited to be linked to seasonal signals that vary by geographical location (Hahn, 2016).

One such plant-based metabolic is ascorbic acid, also known as Vitamin C. Ascorbic acid is an essential part of daily human nutritional requirements. Adults need 75-90 mg of vitamin C per day, as recommended by the Food and Nutrition Board at the Institute of Medicine of the International Academies of Sciences. When looking at total body content of Vitamin C, an adult human can have anywhere from 300mg (levels that would be near scurvy) to around 2g. The body tightly controls the absorption of vitamin C, such that people can consume large amounts of the vitamin, only to excrete the excess in the urine (NIH, 2018). Ascorbic acid functions within the body as a facilitator in the biosynthesis of collagen, protein metabolism, and as a physiological antioxidant. It also has an important role in bolstering the immune system and potentially staving off the onset of certain cancers (NIH, 2018). Vitamin C supplements are available on the market, but long term storage of such supplements can lead to degradation of active ascorbic acid content. According to a 2006 paper on vitamin C

degradation, vitamin C degrades first by oxidizing into dehydroascorbic acid, and then further into diketogulonic, oxalic, and threonic acids. While the first reaction is reversible, the next ones are not. This is the mechanism by which vitamin C is “lost” over time in prepared or stored food or supplements. It is also very sensitive to heat conditions, light, oxygen exposure, and pH conditions. Irradiation or pasteurization may also destroy vitamin C. The rate of degradation of vitamin C is dependent on these factors and not an independent degradation factor of vitamin C itself. With this in mind, investigation into methods for producing consistent sources of fresh ascorbic acid become more important when access to nutritional supplements becomes limited.

Consistent fresh vitamin C production relies on an understanding of the biological pathways involved. Ascorbic acid pathways have been studied and are well described in plants (Wheeler et al, 1998). Specific carbon compounds and enzymes involved in at least one pathway have been identified. L-galactose and D-mannose in particular have been found to be intimately involved in the production of L-ascorbate, which is an alternate and usable form of ascorbic acid (Wheeler et al, 1998). The activities of the various photoreceptors are less well understood, but a general understanding has been reached for several classes (Folta & Childers, 2008; Massa et al, 2008; Galvao & Frankhauser, 2015).

UV light is electromagnetic radiation with wavelengths between 100 and 400 nm. UVC light ranges from 100-280nm. UVB ranges from 280-315nm. UVC ranges from 315-400nm. 400 nm signals the beginning of the visible spectrum with violet and blue light ranging from 400-500nm. as previously discussed, plants are able to use blue light for photosynthesis, so it is not so much of a stretch to imagine that nearby

wavelengths may also be useable. However, UV light comes with many drawbacks. Prolonged exposure to UV light damages both plants and animals. In plants, this can result in DNA damage and lesions, though it also may trigger responses via gene activations, and these effects can occur potentially within hours (Mpoloka, 2008). Cost is another factor, with different wavebands tending differ in cost depending on mechanical requirements. Worker safety must also be taken into consideration as exposure to UV light is just as damaging for humans as it is for plants.

The beneficial effects of UV light have already been investigated in previous studies, however nothing significant was found in the areas of antioxidant (ascorbic acid) production. Given that response to UV light depends upon cryptochrome photoreceptors, this study aims to investigate how blue light may affect ascorbic acid production, flowering time, fruit production, and seedling height in Micro Toms.

Methods and Materials

An experiment was conducted with 4 lighting treatments set up in two controlled environment chambers (dimensions 8’W x 12’L x 7’H). The experiments used tomato ‘Micro Tom’, selected both for its small growth habit and for its commercial availability

Table 1: Lighting treatments per physical lighting system by replicate number. Physical lights are represented by L1-L4. Lights L1 and L2 were hung in one chamber and L3 and L4 were hung in a second chamber Rep 1-3 indicates during which replicate the physical light had which lighting treatment applied.

Rep	L1	L2	L3	L4
1	55R:45B	70R:30B	90R:10B	Control
2	90R:10B	55R:45B	Control	70R:30B
3	Control	90R:10B	70R:30B	55R:45B

(seeds were from PanAmerican Seed, West Chicago, Illinois). Four custom built LED structures were ordered specifically for our growth

chambers and this project. The lighting fixtures were designed with the lighting

capabilities of NASA's Advanced Plant Habitat (APH) in mind. Five single-color arrays were individually controllable using 10 power supplies (HLG-150H-36B and HLG-320H-24B, Mean Well, Taiwan) for finely tuned spectral environments: Blue, Green, Red, Far-Red, and White (440 nm, 520nm, 630nm, 740 nm, and 4100K). Each of the four fixtures was electrically independent but shares a water-cooling system, keeping water temperatures consistently near 100 °F.

Within each controlled environment chamber, two areas were set up for lighting treatments resulting in space for 4 different LED treatments (physical space is denoted as L1, L2, L3, and L4). The lighting treatments made use of the blue, red, and white diodes with power supplies adjusted to supply light ratios as noted in Table 2.1. Light intensity under each treatment averaged $300 \pm 5 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Each lighting system had a timer, and lights were on for 14 hours per day, which resulted in achieving a daily light integral (DLI) of $15.12 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$. The experiment was repeated over time for a total of three replicates with. Light treatment was rotated across each replicate crop cycle as noted in table 1. The growth chamber air temperature set points were 25/21 °C day/night temperatures. The day period ran from 8 am to 10 pm, with night running from 10 pm to 8 am. Some differences in air temperature were observed over seasons, potentially due to mechanical differences in cooling capabilities of each chamber. The lighting systems (as described above) were hung from the ceiling of each chamber. Plants were placed on a table centered under each light fixture and lights were 2 feet above average plant canopy height). Between the two light fixtures in each growth chamber a reflective foam barrier was erected to reduce light spillover between

treatments. Even with the barrier in place, light spillover varied from 1 to 5 $\mu\text{mol}/\text{m}^2\cdot\text{s}^1$, depending on measurement point.

For each of the three replicate crop cycles tomatoes were seeded into 72 cell flats filled with Cornell soilless media and germinated in greenhouse conditions under sunlight and high-pressure sodium lights. Seedlings remained in greenhouse for 1-2 weeks until all seedlings had germinated and developed the first set of true leaves before being moved into the growth chambers for their respective treatments. At this point, trays were cut in half to provide 36 cells per treatment. After about 2.5 weeks under lighting treatments 32 seedlings per treatment were transplanted into 4-inch (500 mL volume) pots with Cornell soilless media, upon development of the first flower set. Plants remained in 4" pots for weeks during which time initial flower and fruit development took place. At 4 weeks after transplanting the first fruit harvest was conducted and 16 plants were selected and transplanted into 6-inch (1700 mL volume) pots with Cornell soilless media. After the initial harvest, fruit harvest was conducted every two weeks. After 4 weeks in the 6" pots (12 weeks after seeding and 9.5 weeks after beginning light treatments), a final fruit harvest was conducted and destructive harvest was used to determine plant fresh and dry mass. At each transplant stage, the healthiest plants are selected for continued growth. At the transplant stage, plants were watered daily to ensure even wetness of plugs with Jack's Professional LX 15-5-15 Calcium + Magnesium LX. This fertilizer was used throughout all stages of plant growth. As plants grew larger and were transplanted. Plants were watered as needed, between every other day and every fourth day.

During the first replicate crop cycle no pest/disease control measures, however, a severe powdery mildew outbreak occurred in the last phase of growth. During the second replicate, Suffoil-X (BioWorks, Inc., Victor, NY), Cease (BioWorks, Inc., Victor, NY), Trigo (Bayer, Research Park Triangle, NC), and Milstop (BioWorks, Inc., Victor, NY) were applied to combat any potential powdery mildew outbreak. Trigo was applied three times, Suffoil-X was applied once, and Cease/Milstop was applied three times. During this replicate, plants were healthiest and no signs of disease or pests were observed. In the third replicate, Cease, Milstop, and Suffoil-X were used three times, and one application of Acephate (United Phosphorus, King of Prussia, PA), Avid (Syngenta, Greensboro, NC), and Conserve (Syngenta, Greensboro, NC), was required to combat a thrips outbreak. Plant health was compromised near the end of the third replicate from pest damage.

During the crop cycles data were collected on several parameters. Plant height measurements (from the soil line to the bottom of the apical bud) were taken of seedlings when they were moved into the chamber, and subsequently each week until they were transplanted into 4" pots. After transplant into 4" pots, the number of flowers and developing fruits per each plant per treatment were counted weekly. For flowers, only fully open flowers were counted. For fruits, any green fruit larger than 3mm were counted developing fruit. Every two weeks, ripe red fruits from each plant were harvested, weighed, and counted. During harvest, only white lighting was used in order to make grading of ripeness more consistent between lighting treatments. Fruit size was calculated as average fruit size based upon total fruit weight for an individual plant and the number of fruit that the plant produced at a given harvest time. From each replicate

1-2 harvests were selected to record Brix. In the first replicate, Brix was measured at the same time as harvest but selecting a ripe fruit from each plant and squeezing juice directly onto the handheld refractometer. This method was refined for the second replicates. In this replicate, tomato fruits were stored overnight in Ziplock baggies, inside a cooler, before measurements. Measurements were conducted by crushing multiple fruits within baggies and squeezing the homogenized juice from the bag onto the refractometer. The refractometer was rinsed and dried between each reading.

Ascorbic Acid Extraction and Measurement

At select harvests in the second and third crop cycle, six plants were selected for analysis from each treatment. A protocol for extraction was prepared based on protocols used in previous literature for ascorbic acid extraction (Fatariah, 2015; Cotrut & Badulescu, 2016). All of the fruits from that plant were taken and ground up using a mortar and pestle to homogenize the fruit samples. 5 g of homogenized fruit was weighed and put into a 50 mL centrifuge tube without solvent. Tubes were placed on ice in a light tight container during the collection process until all 24 samples were prepared. For the first two ascorbic acid preparations, metaphosphoric acid solution was prepared as the extraction solution. In the first replicate, 9% metaphosphoric acid (MPA) solution was used. In the second replicate, 3% MPA solution was used, with water as an extraction agent in several samples for side-by-side comparison of effectiveness. In the third replicate, water was used as the extraction agent after it was determined that MPA solution vs. water extraction did not impact results. After the 5 g samples were ready, 15 mL of extraction solution was added. All samples were vortexed for 5 seconds and placed in a 4 °C fridge in their light tight container for 45 minutes for

the extraction period. After the extraction period, the 50 mL tubes were centrifuged for 10 minutes at 4000 rpm, and 10 mL of the supernatant was transferred to 15mL tubes for transport to the analysis lab. Prior to analysis, dithiothreitol (DTT) was added to each sample. Samples were analyzed using HPLC and measuring absorption at 254nm. The experiment was set up as a completely randomized design and was repeated over time for a total of three replicates. All data was analyzed using R to develop ANOVA tables and mixed effect models that account for treatments, random effects of plants, and fixed chamber effects. The number of replicate plants under each light treatment varied during the crop stage (and was 36 for seedling stage, 32 for 4-inch stage, and 16 for 6-inch stage).

Results

Seedling Height

Regarding seedling height, there was little difference in height between lighting treatments in the first two weeks of exposure to treatments. Using a Tukey's HSD, two groups were found at time point three, two weeks after exposure to treatments. Control plants were distinct from 55:45 treatment plants. Control plants were taller than all treatments, but significantly taller than the 55:45 treatment. Table 2.2 contains a summary of raw seedling height averages, while table 2.3 contains the results of our statistical analysis of seedling height over time.

Table 2: Seedling height in centimeters. Measurements of seedlings were taken every seven days for the first two weeks in the chamber.

	Days in Treatment	Height (cm)			
		Control	55R:45B	70R:30B	90R:10B
Exp 1	1	2.54	2.71	2.93	2.57
	8	4.56	3.69	3.66	3.89
Exp 2	1	2.43	2.91	2.79	3.01
	8	3.61	3.66	4.14	4.02
	15	4.94	4.79	5.47	5.70
Exp 3	1	2.58	2.41	2.69	2.15
	8	4.39	3.11	3.52	4.05
	15	6.47	5.59	5.83	5.96

Table 3: Seedling height statistical means and statistical significance by time points. Time point 1 represents day 1, time point 2 day 8, and timepoint 3 day 15. A Tukey's HSD was performed to further clarify relationships between treatments.

Day 1			Day 8			Day 15		
Treatment	Mean +/- SE	Tukey's	Treatment	Mean +/- SE	Tukey's	Treatment	Mean +/- SE	Tukey's
Control	2.53 +/- 0.17	A	Control	4.23 +/- 0.17	A	Control	5.81 +/- 0.19	B
55R:45B	2.64 +/- 0.17	A	55R:45B	3.47 +/- 0.17	A	55R:45B	5.21 +/- 0.19	A
70R:30B	2.79 +/- 0.17	A	70R:30B	3.73 +/- 0.17	A	70R:30B	5.63 +/- 0.19	AB
90R:10B	2.61 +/- 0.17	A	90R:10B	4.00 +/- 0.17	A	90R:10B	5.83 +/- 0.19	AB

Developing Flowers

At 47 and 54 days there were no significant differences in flower number by but by day 65, there were significant differences in mature flowers as the number of mature flowers decreased and developing fruits increased. At day 65 the control and 90:10 R:B treatment had about two more flowers per plant than the 70:30 and 55:45 treatments (Table 2.4).

Table 4: Developing flowers, means \pm standard error reported in number of fruit in replicate 3. Tukey's HSD identified two separate groupings.

Age = 47:			Age = 54:			Age = 65:		
Treatment	Flowers	Tukey's	Treatment	Flowers	Tukey's	Treatment	Flowers	Tukey's
Control	6.5 \pm 0.28	A	Control	6.13 \pm 0.07	A	Control	4.81 \pm 0.06	B
55R:45B	5 \pm 0.22	A	55R:45B	5.37 \pm 0.06	A	55R:45B	2.66 \pm 0.11	A
70R:30B	4.17 \pm 0.2	A	70R:30B	4.87 \pm 0.06	A	70R:30B	2.3 \pm 0.12	A
90R:10B	4.5 \pm 0.24	A	90R:10B	5.57 \pm 0.08	A	90R:10B	4.38 \pm 0.06	B

Developing Fruits

Prior to reaching the red harvestable stage, the number of developing fruit per plant were recorded. At 47 days under treatment here were no significant differences. At day 54, the Control treatment had about two more developing fruit per plant than the 90:10 and 55:45 R:B treatments. By day 65, the 70:30 and 55:45 R:B treatments had significant more fruit (about 4 more) than the control and 90:10 R:B treatments (Table 2.5)

Table 5: Developing fruit means \pm standard error, reported in number of fruit in replicate 3. Tukey's HSD identified two separate groupings.

Age = 47:			Age = 54:			Age = 65:		
Treatment	Fruits	Tukey's	Treatment	Fruits	Tukey's	Treatment	Fruits	Tukey's
Control	2.5 \pm 0.45	A	Control	10.9 \pm 0.06	B	Control	12.17 \pm 0.04	A
55R:45B	6.5 \pm 0.2	A	55R:45B	8.05 \pm 0.05	A	55R:45B	15.63 \pm 0.04	B
70R:30B	6.5 \pm 0.16	A	70R:30B	8.31 \pm 0.05	A	70R:30B	16.67 \pm 0.04	B
90R:10B	6.75 \pm 0.19	A	90R:10B	9.5 \pm 0.06	AB	90R:10B	11.22 \pm 0.04	A

Fruit Yield

There were no significant differences in mean cumulative fruit weight per plant (Table 2.6). However there appeared to be a pattern whereby 90:10 R:B led to the highest fruit weight per plant, followed by 55:45 R:B, followed by control.

Table 6: Fruit yield in total fresh weight of fruit per plant (grams). No Tukey's HSD was performed due to lack of statistically significant differences in means between treatments.

Treatment	Weight (g)
Control	19.33 \pm 3.71
55R:45B	19.73 \pm 3.71
70R:30B	17.92 \pm 3.71
90R:10B	21.49 \pm 3.72

Regarding the number of fruit per plant the 90:10 R:B treatment had a greater number of fruit per plant (6.3) than the 70:30 R:B treatment (4.9) (Table 2.7).

Table 7: Number of fruit, with means \pm standard error. A Tukey's HSD was performed and two groups were identified.

Treatment	Tomato No.	Tukey's
Control	1.72 \pm 0.07	AB
55R:45B	1.76 \pm 0.07	AB
70R:30B	1.64 \pm 0.07	A
90R:10B	1.87 \pm 0.07	B

There were no significant differences in mean weight of individual fruit (Table 2.8).

Table 8: Mean weight of individual fruit as calculated by total harvest weight / number of fruits harvested, per plant. Means (in grams) \pm standard error reported. Tukey's HSD omitted due to lack of statistical significance.

Treatment	Average Fruit FW(g)
Control	3.5 \pm 0.58
55R:45B	3.54 \pm 0.58
70R:30B	3.41 \pm 0.58
90R:10B	3.52 \pm 0.58

There were no significant differences in Brix of tomato fruit, and these varied by treatment from 4.64 to 5.19 °Brix (Table 2.9).

Table 9: Mean °Brix \pm standard error. Tukey's HSD omitted due to lack of statistical significance.

Treatment	°Brix
Control	4.64 \pm 0.65
55R:45B	5.19 \pm 0.65
70R:30B	4.56 \pm 0.65
90R:10B	4.42 \pm 0.65

Ascorbic Acid Extraction and Measurement

In crop cycle 2, relative ascorbic acid analysis was performed by the Cornell Core Life Sciences facility. Data are in arbitrary units as a standard was not available. There were no significant differences by treatment due to small sample size (n=6 per treatment) and high variability, but an interesting pattern emerged whereby 70:30 R:B and control exhibited greater ascorbic acid content than 55:45 R:B or 90:10 R:B (Figure

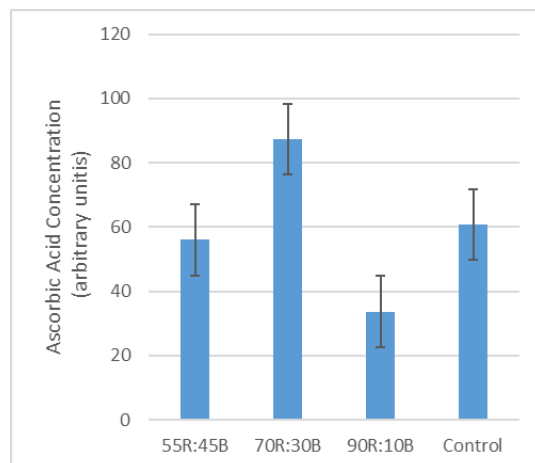
2.1). Overall, treatments appeared to have large enough differences to warrant further investigation.

In crop cycle 3, two ascorbic acid extractions and analyses were performed, this time by the Morau lab in Food Science and with a true standard. No significant differences in ascorbic acid concentration were observed and this varied from 77 $\mu\text{g/mL}$ for the control to 81.59 $\mu\text{g/mL}$ for 55:45 R:B (Table 2.10)

Table 10: Mean ascorbic acid content \pm standard error, reported in $\mu\text{g/mL}$.

Treatment	Ascorbic acid($\mu\text{g/mL}$)
Control	77.69 \pm 3.11
55R:45B	81.59 \pm 3.11
70R:30B	79.83 \pm 3.11
90R:10B	81.41 \pm 3.11

Figure 1: Average Relative Ascorbic Acid. Arbitrary units reported in order to compare relative concentrations.



Discussion

Regarding seedling height, varying reports of plant responses have been made. When grown under monochromatic blue light, internode length of cherry tomato seedling is reported to have increased approximately 2 times that of seedlings grown under monochromatic light (Kim et al., 2014). Under combinations of lighting treatments, it was found that treatments containing 25% or more blue light produced more compact seedlings than other treatments (Wollaeger & Runkle, 2014). Both

studies would indicate that leaving blue light out of the spectrum entirely may produce taller plants or simply not elicit the compactness tendency. Wollaeger and Runkle note that “extremely low-fluence B light [...] can stimulate phototropins and cause an increase in extension growth...”, therefore pointing to phototropin activity as the likely source of the results of the Kim et al. study. The present study provided blue light at thresholds greater than those indicated by these previous studies and avoided the issue all together. Giliberto, et al. (2005) found a response of shorter seedlings in response to blue light with overexpression of blue light receptors, and Hernandez et al. (2016) found a decrease in seedling height with an increase in blue light percentage applied to plants. These previous results may have suggested a more strongly significant response in seedling height, though this may be due to the fact that seedlings in the current study were germinated outside of the treatments in a uniform spectral environment.

Fruit in tomato plants have been shown to have measurable responses to lighting treatments (Punjai et al, 2017; Castagna et al. 2014 Dazkovich et al., 2016). Punjai et al. found that post-harvest treatments with UV and red light increased lycopene concentrations and decreased ripening time. Castagna et al. found that UV-B light specifically elicited a response of increased antioxidant activity in both the peel and flesh of fruits. A brief review of reactive oxygen species (ROS) and plant defenses given by Kim et al. (2014) explains that plant defense mechanisms or stress responses may be triggered by changes in environment as ROS accumulate. These stresses responses often take the form of antioxidant substrates, and measuring antioxidant capacity may be a way to measure stress levels of plants. Ascorbic acid is tightly involved in response to environmental stresses such as low temperatures, drought, or salt stresses, and an

increase in ascorbate content and Asc recycling in tobacco plants lead to increased tolerance to these conditions (Eltayeb et al., 2006; Gallie, 2012). The subject of the study performed by Kim, et al., was also lighting treatments, and they found that antioxidant capacity was significantly higher in seedlings grown under monochromatic blue light. This could indicate that blue light invoked a stress-like response similar to that of UV light. It was unfortunate that no strong conclusions could be drawn from the current study's ascorbic acid data. In our plants, we measured ascorbic acid concentration in the fruits, and a number of stress-factors may have contributed to obscure true trends from our results. Given the pest pressures and nutrient deficiency symptoms plants were experiencing, a background level of stress was present among all treatments, potentially skewing results.

As for flower count, blue light has been found to be a factor influencing flower regulation. Wollaeger & Runkle found that, in *impatiens* plants, increasing amounts of blue light lead to increasing numbers of flower buds at harvest (2014). In the same study, tomatoes were also investigated, but flowers had not developed at harvest time. Wollaeger and Runkle postulate that the photoreceptor CRY2 cryptochrome may be responsible for this response in *impatiens*, as it is involved in regulation of flowering response in photoperiodic plants. CRY1 and CRY2 have both been implicated in control of flowering time in *Arabidopsis* (Giliberto, 2005; Yu 2010). The significance of flower count response and thus developing fruit response in the current study implies that tomato flowering is also susceptible to blue light as a method of manipulation. With the current study, higher blue light led to a decline in number of flowers sooner than lower blue light treatments. Changes in the number of flowers appeared to correlate with a

change in the number of fruits developing overall as well. Combined with the higher number of developing fruits throughout the measurement period, it may be inferred that either plants began developing flowers and fruits sooner, or development initiated at the same time and simply progressed faster. More studies could be conducted to further elucidate this difference.

Several experimental shortcomings limit the ability to interpret the experimental results. Depending on crop cycle there were several plant disease and health issues and these were sometimes more prominent in one controlled environment chamber than the other. With the benefit of hindsight I can recommend a more proactive pest and disease management approach for future work. Such an approach would involve: more frequent scouting for pest/disease (perhaps three times a week rather than once per week); use of beneficial insects for preventative thrips control such as deployment of sachets containing the beneficial mite, cucumeris, to control thrips; and for powdery mildew weekly preventative sprays of Cease (a beneficial bacteria) and Milstop (a potassium bicarbonate product). In addition future work should consider greater isolation of growth chambers and allow access only to trained staff that have taken appropriate hygiene measures (disinfecting baths for footwear, Tyvek suits, and hair nets). Regarding data collection, there was some inconsistencies in the data that was collected in each crop cycle. This was due to tomatoes and Micro Tom, in particular, being a new experimental crop in our research group and learning from the initial crop cycle which measurement parameters were most important. As well, in some cases data were collected at different dates within the crop cycle based on differences in plant growth and time to flowering/fruitletting between crop cycles. The methodology to extract a fruit

sample for Brix measurements, also required refinement from the first crop cycle to settle upon a reliable method. Finally, there was some non-uniformity in PAR distribution within treatments. This non-uniformity was due to the nature of LED lights themselves as well as the shape of the fixtures used (which used fairly widely spaced point source diodes for each waveband). In the future, a schedule for rotating plants through different positions on the lights, could homogenize plant exposure to PAR and light quality.

Conclusions

Overall, there were not huge differences in plant yield or quality (Brix, ascorbic acid content) in response to R:B lighting treatments. The relatively minor response of the plant to lighting treatment may be due, in part, to the specific cultivar. Micro Tom is a dwarf variety, and the overall small size and determinate nature of the plant may limit its morphological plasticity. In some measures (list) the 70:30 R:B treatment exhibited reduced performance as compared to the 90:10 or 70:30 treatment. More research is required to determine if this trend applies to other tomato varieties.

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CHAPTER 3

A PROPOSED CURRICULUM FOR MIDDLE SCHOOL SCIENCE EDUCATION USING PLANT SCIENCE AS THE BASIS FOR SCIENTIFIC INQUIRY

Introduction

It may seem like, given the amount of information presented in the first two chapters, comprehension of specialized plant science topics requires quite a bit of background knowledge. There is indeed a large amount of study that goes into understanding and identifying gaps in current research, which only a small segment of the general population has any real interest in pursuing. This does not mean that this information should not be accessible or have an impact on people outside of the field. But how can we make current research seem less obscure and more relatable to a larger number of people? Specifically, the driving question behind this chapter is “How do we get the next generation interested in plant science, or science in general?” The answer to this question can take many forms and imply a whole host of other questions in and of itself. In this instance, I have chosen to answer this question through improving and supplementing the science education of the next generation of scientists and agriculturalists.

Education in America is a hot topic, rife with criticism and praise from many sources. The Programme for International Student Assessment ranked the United States 38th out of 71 countries when assessing for math performance at age 15, and 24th when assessing science performance (Pew Research, 2017). This is not the only testing method that ranks the United States in the bottom half of performance metrics. The Trends in International Mathematics and Science Study, as well as the National

Assessment of Education Progress, have been similarly tracking American student performance in math and science since the mid 90s. Science knowledge is important for all Americans in our increasingly technology-based world, and science education makes this knowledge available from the earliest stages of learning. But there are many ways to effectively teach science, and plant science can be an effective and engaging framework upon which to base learning activities. Following current science education standards, this chapter proposes a set of plant-science-based learning activities for 7th graders to improve understanding of the science behind plant growth, awareness of growth requirements and the effects of the environment, and encourage interest in continuing education or careers in Agriculture and Plant Science.

According to the National Center for Education Statistics, the trend in Bachelor's degree conferral for biological and biomedical science degrees is in a slow but upward trend. Biological and biomedical science degrees still make up only a small portion of the total number, under 10% (Undergraduate degree fields, 2018). This percentage encompasses degrees that are unrelated to plant sciences. Further breaking these numbers down, in 2012, 30,929 Bachelor's degrees were conferred in Agricultural sciences, an area that encompassed plant science and other related degrees. Out of a total of 1,791,046 Bachelor's degrees conferred by postsecondary institutions in the United States in 2012, this makes up a total of 1.7% of total degrees conferred being in Agricultural Sciences (Digest, 2012). This number seems woefully inadequate in the face of the importance of Agriculture within the United States and the world. According to the USDA, \$992 billion was contributed to the US gross domestic product (GDP) in 2015 by agriculture activities and related industry, which made up a 5.5% share of the

total GDP. 11% of total US employment is made up of agricultural related jobs (USDA, 2018). Opportunities abound in the Agricultural sector, though many challenges are facing today's generation of agriculturalists. These challenges would be well served by a future generation of well-educated, science-minded farmers; the problem becomes, how do we generate interest and effectively educate this next generation?

Science education has undergone a multitude of changes over the years. The National Science Education Standards (NSES), developed and established by the National Research Council in 1996, had considerable influence nationwide when it comes to state-level implementation of science curriculum (Herr, 2007). The NSES stated that “all students should develop abilities necessary to do scientific inquiry,” and “understandings about scientific inquiry” (NSES, 143). In the best of circumstances, students had the opportunity to participate in “full inquiries” where a full scientific investigation is performed, from forming a hypothesis, to testing it, gathering data, and presenting their conclusions. These presentations may take the form of oral or written reports, and the NSES suggested there be multiple opportunities given for such reporting activities. At this stage of science education, emphasis was placed on students receiving feedback on the quality of their thoughts and expressions. The NSES's standards for Life Sciences content mandated that students develop an understanding of “structure and function of living systems; reproduction and heredity; regulation and behavior; populations and ecosystems; diversity and adaptations of organisms” (Content Standards, 155).

The Next Generation Science Standards further develops and expands the NSES curriculum guidelines and is the set of standards currently in use. It provides curriculum

targets beginning in kindergarten, using both plants and animals as examples of life and the basic activities and functions of living organisms: reproduction, food and water cycles, diversity of life, and matter and energy flow. This framework of science education allows for a wide range of topics to be covered and put into appropriate context. According to these standards, in middle school, students should begin to learn how to form scientific hypotheses and carry out investigations to help them determine the validity of their hypotheses (NGSS, 2013). Using this set of standards, teachers and curriculum designers are provided with a roadmap of where their students should be at each age range while allowing creators some flexibility in where their focus lies in the details of the curriculum supplement.

Given a clearly defined set of standards and goals, curricula may be developed using a wide range of subject matter for demonstration and interaction. For example, plants are an ideal way to study how organisms self-regulate and react to their environments. Plant science is an area of biology that focuses on the study of plant systems, from growth and development, to evolution, and their interactions with the environment around them. Plants grown in or near the classroom give students an opportunity to physically examine and interact with the subject of their study, an opportunity that is perhaps not afforded during the study of larger animals or humans for practical or ethical reasons.

Plants are often the “forgotten half” of biology (Mattson, personal communication). Though Plant Science degrees are growing increasingly popular, this by no means puts them into competition with other sciences or even non-science-based degrees. How can this situation be remedied? By increasing exposure of the younger

generation to a wider range of topics, showing that science can be interesting in a number of ways. Personally, I had a hard time connecting to science as I was growing up. What we were learning didn't seem useful to me in general, as they were topics I didn't care much for or didn't see the purpose in my everyday life. Children in middle school and elementary school learn about very large topics, such as Earth Sciences and the generalities of large ecosystems., or very small topics such as the structure of function of cells. While these are still very important topics, it can be hard to place themselves into a context that makes this science relatable to them. Plant Science, and in particular the study of food crops, makes science immediately relatable by putting tools into children's hands to immediately begin growing their own plants and investigating science for themselves, directly observing effects that they've learned about in class.

Beyond Plant Science, there is a lack of understanding of the effects of light on plants in both industry growers and producers of horticultural lights. This is potentially due to the fact that photobiology is a field that is not as well established as other components of horticultural study. The ability to study the effects of specific wavebands of light on plants has developed only as quickly as our ability to produce LEDs with specific wavebands. Progress has been made academically, but it takes time for this knowledge to become accessible to relevant industry people both in terms of publication restriction and the ability to understand. As a graduate student, I have interacted with a number of representatives of companies in the indoor agriculture business. Some of these individuals are either manufacturers of lighting systems or sales people for those companies; I have also met a number of growers of many different types of crops.

Invariably, these growers have been interested to learn more about LEDs and lighting effects, while still employing industry standards of high-pressure sodium lamps and incandescent bulbs, depending on crop type and production cycle. Some growers have begun to include LEDs in their growing equipment, but different manufacturers often provide different base settings or recommendations for their light fixtures.

Without a familiarity with plant science in an academic setting, the resources to make strong decisions are thin. LEDs offer an incredible amount of flexibility in terms of light spectrum and electrical efficacy; with such a range of ability comes the problem of deciding what features are best for your crop in particular. Should a grower stick with a 20% blue and 80% red LED fixture? Do they really need that much blue? With red being more electrically efficient, maybe they should decrease the amount of blue in their spectrum, but what effect will this have on the plants? In order to understand these lights and their effects, growers currently look to manufacturers to provide fact sheets, demonstrations, and consultations on the appropriate use of their products without having many other sources of data or context. With a stronger focus on plant science and an understanding or even simple awareness of photobiology from a younger age, a new generation of consumers will be armed with critical thinking tools and contextual knowledge to enable them to become more effective growers and potentially sharper, more curious scientists.

Given these criteria of what to focus on (plant science, understanding of photobiology), a curriculum supplement was developed with the aim being to engage middle school students in scientific thought and spark interest in plants as an area of study. This proposed curriculum supplement, “Why isn’t my lettuce red?”, provides a

set of learning activities and investigations centered around plant science. This supplement includes many of the goals of both the NGSS and NSES, specifically allowing students to become familiar with the idea of proposing answers to questions they can then investigate scientifically. Students are presented with an initial situation and question, then over the course of several weeks are given access to resources where they may gather relevant information and propose and even modify hypotheses as they continue to gather data. Students may feel ownership over the data they are gathering through direct contact with the subject of their study, the growing lettuce seedlings.

The use of red lettuce in particular gives students a front row seat to seeing how plant adaptations are dynamic and environment dependent. Red lettuce varieties are eye catching and gaining popularity. Their growth and form are identical to the more traditional green varieties, but their morphology is such that they develop red pigments called anthocyanins in a variety of patterns in response to high light intensities or the presence of blue light. An awareness of the environment becomes a segue into a discussion of larger ecosystems and where such plants might flourish or perish. Another logical extension of such a curriculum is growing plants out beyond their harvest stage to the reproductive stage and learning about plant-based reproduction.

The study of food crops gives educators a unique opportunity to bring the study of science into a context that is intimately familiar to students. Most students are familiar with food crops, specifically lettuce, for its use in salads, sandwiches, and many other common food items. In the United States, per capita consumption of lettuce was 12.5 lbs in 2017 (USDA ERS, 2018). Not many students are given the opportunity to see lettuce production on a large or even small scale, outside of a family or community

garden. Large scale lettuce production typically happens in California, Arizona, and Mexico, with smaller greenhouse-based operations throughout the US. The availability or accessibility of such resources cannot be counted upon to give children an understanding of where their food comes from. By putting living food crops into the classroom, students will gain a better understanding of where their food comes from. By encouraging children to eat the food they have grown, they will gain a sense of ownership over their work and accomplishments.

Beyond the plant science focus, the use of light-emitting diode (LED) lights in the classroom adds a technology aspect to the project that provides valuable experience and thought-provoking experiences. LEDs have been around for decades, but within the last decade or so have gained attention for practical applications in horticultural settings. The compactness and versatility of this technology makes them ideal for photobiological (the study of light's effect on biological processes) studies. High tech, sophisticated horticultural lighting systems employing LEDs are currently available in a variety of forms, but tend to be expensive. Photosynthetically relevant levels of light can be produced on a much smaller scale with commercially available equipment, enabling educators to perform small-scale demonstrations of photobiological reactions right in the classroom.

The proposed curriculum supplement offers students the opportunity to be introduced to scientific thinking and questions, data collection and scientific reporting, a framework for understanding plant form, function, and requirements, and knowledge of where the food that they eat on a daily basis comes from. Students gain an understanding of the nature of the environment around them and how their environment

can have real, immediate impacts on organisms, while also being introduced to different paths of study where they can learn more about these topics.

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Appendix I: Curriculum Supplement

Why isn't my lettuce red?

A plant science exploration

Erica Hernandez, MS, Jeff Perry, PhD.
Cornell University, 2019.

Summary

Using a series of hands-on activities, worksheets, reading assignments, and mini-lectures, middle school students explore a variety of topics associated with plant science research. This curriculum is intended to be used on an every-other-day schedule for a period of four weeks in addition to other existing science curricula.

Pre-req knowledge

Students should have some basic understandings of water and light from previous science classes, and a general understanding of requirements for life in different organisms.

Learning Objectives

After completing this series of activities, students will:

- Understand and be able to apply the scientific method.
- Have experience collecting, recording, and analyzing data
- Be able to provide basic plant care
- Be familiar with basic biological processes occurring in plants.
- Be able to use library and internet resources to answer questions.
- Be able to apply critical thinking
- Have an awareness of the importance of the environment.
- Have an idea about Plant Science careers.
- Become familiar with how plant-based food is grown and processed.

Educational Standards

This series of activities meets NGSS Science Standards for content for 7th grade, focused primarily on Structures and Processes of Organisms, and Organization for Matter and Energy Flow in Organisms:

- Developing models to describe unobservable mechanisms (MS-LS1-7)
- Constructing scientific explanations based on valid and reliable evidence obtained from sources (including the students' own experiments) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future (MS-LS1-6)
- Science knowledge is based upon logical connections between evidence and explanations. (MS-LS1-6)
- Plants, algae (including phytoplankton), and many microorganisms use the energy from light to make sugars (food) from carbon dioxide from the atmosphere and water through the process of photosynthesis, which also releases oxygen. These sugars can be used immediately or stored for growth or later use. (MS-LS1-6)
- The chemical reaction by which plants produce complex food molecules (sugars) requires an energy input (i.e., from sunlight) to occur. In this reaction, carbon dioxide and water combine to form carbon-based organic molecules and release oxygen. (*secondary to MS-LS1-6*)
- Cellular respiration in plants and animals involve chemical reactions with oxygen that release stored energy. In these processes, complex molecules containing carbon react with oxygen to produce carbon dioxide and other materials. (*secondary to MS-LS1-7*)
- Analyze and interpret data to provide evidence for phenomena. (MS-LS2-1)

- Construct an explanation that includes qualitative or quantitative relationships between variables that predict phenomena. (MS-LS2-2)
- Construct an oral and written argument supported by empirical evidence and scientific reasoning to support or refute an explanation or a model for a phenomenon or a solution to a problem. (MS-LS2-4)
- Science disciplines share common rules of obtaining and evaluating empirical evidence. (MS-LS2-4)
- Science findings are frequently revised and/or reinterpreted based on new evidence. (MS-ESS2-3)

Project Set Up

Title: LED Box Setup

Grade Level: Teacher (7)

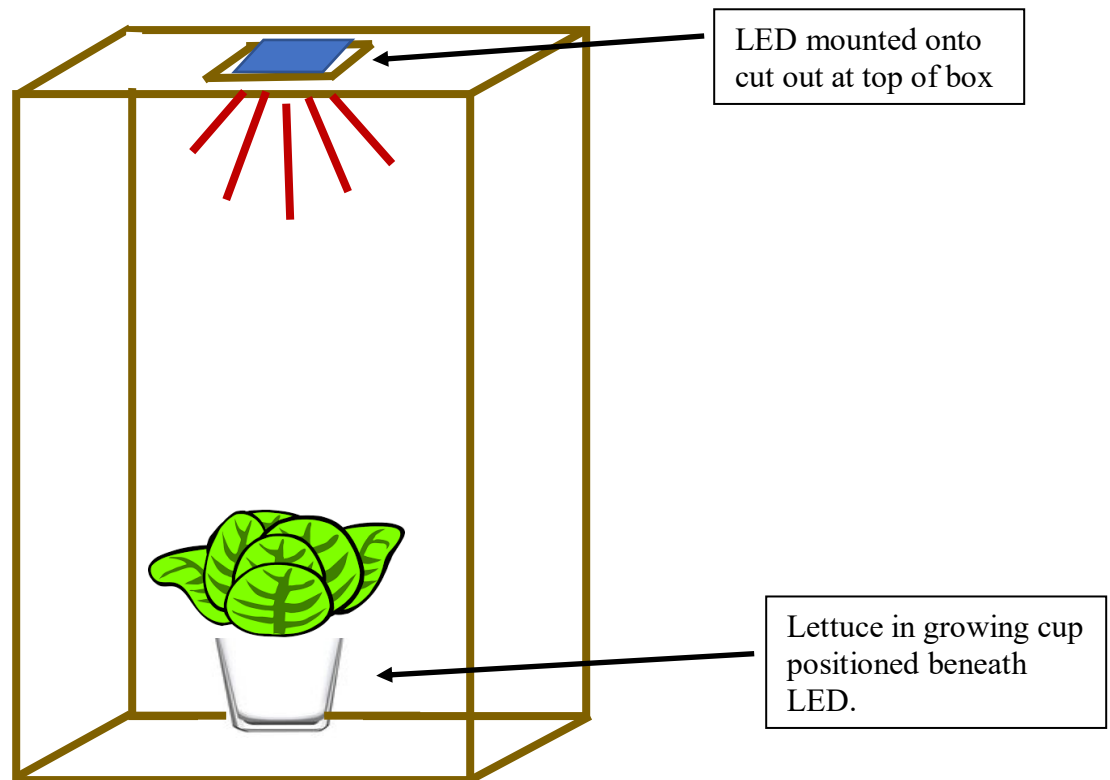
Subject: Electronics assembly

Objective: To correctly assemble the shoebox photosynthesis setup.

Activities:

During this time, the teacher will set up two shoe boxes with attached LED arrays, following the included instructions. Each shoe box should have a different color of LED light. Once assembled, the teacher will maintain one lettuce seedling per box, and use them as example plants during activities and rank them as part of the class data collection. Around day 21, lettuce plants will switch boxes to demonstrate the effects of light environment on plant growth response.

- 1) Ensure that your voltage and amp draw on each LED match the voltage and amp capacity of your power supplies. Ask an electronics store employee for help confirming this if you are unsure.
- 2) Using solderless twist connectors of appropriate size, connect each LED to a power supply.
- 3) In each shoebox, turn the open side to face you and rotate so they are standing on the short ends.



- 4) Cut a hole in the top of the shoebox in the appropriate size and shape to accommodate the LED board you have selected.
- 5) After your LEDs have been wired to power supplies, LEDs must be mounted across the LED hole on the top of the shoebox. A red LED should be used in one box; a blue LED should be used in the other. Take one or two popsicle sticks and glue them across the back of the LED board as appropriate, avoiding wire connections. The popsicle sticks will hold the LEDs in place while allowing heat to escape from the back of the board.
- 6) Program your timers to follow roughly the light schedule that the rest of the classroom plants will receive. For example, if the lights are on 8 hours a day in the classroom, the timers should be programmed to be on during those hours and off for the rest of the day.
- 7) Lessons will be using these shoeboxes directly for comparison to the student lettuce plants.

Lettuce experiment details: Growing lettuce in the classroom to determine how environment affects size and color. Students seed lettuce into their Jiffy pellets and place all the cups together as the lettuce seeds germinate. In 2-3 days, students will choose a location around the classroom for their lettuce to grow. Students should think about light sources and temperature. The lettuce seedlings will remain in their chosen spot for the duration of the experiment (~28 days). These seedlings will be directly contrasted with the teacher-grown lettuce seedlings inside the shoeboxes, grown under different colors of LED light.

Hypothesis: lettuce seedlings will grow differently in different spots around the classroom. Some will be larger or smaller. Some will be redder than others. This can help students identify the differences between the garden center and the girl's windowsill.

Data to be collected: pictures to record redness of seedlings; Students should draw or take pictures of their plants in place to record progress of growth and redness. Leaf count and head measurement; students should record number of leaves and either height or width (by using a ruler and measuring widest point from leaf tip to leaf tip). These measurements will allow students to become familiar with plant development and growth over time, using measurements that scientists use in the field.

Summaries and comparisons: To summarize their data, students should be able to make statements such as “my lettuce seedling developed 4 leaves in three weeks and grew 1 inch the first week, 2 inches the second week, and 4 inches the third week”. To compare data, students will participate in ranking activities and make statements such as “my lettuce was the 5th largest head but the 10th reddest head”, comparing their heads to other student heads.

Conclusions: Students should be able to take the data they gathered and make a statement about the quality of growth in the spot they picked. They should be able to use the ranking exercises to compare their growing spots to other students' in terms of quality of growth. We would see statements like “my growing spot did not lead to good quality growth when compared to the rest of the class's lettuce heads.” At this point, they will also revisit their hypothesis and either revise either with further guesses about

why their lettuce did/did not grow well, or simply state why it didn't. They can then use this knowledge to guess why the red lettuce was green in the first girl's scenario.

Background Info:

Materials:

Small 660 nm LED, for example <https://goo.gl/Ed6CPr>

Small 440 nm cob LED

LEDs can be obtained at hardware and electronics stores. 12V LEDs are appropriate for this activity

Power-supply for each LED board or strip.

Two-plug timer, for example <https://goo.gl/XAVXc5> (this is a bit expensive but a good example)

Two shoe boxes

Twist on wire connectors: <https://goo.gl/Bk2LFq>

Four popsicle sticks

Lesson 1

Title: Plants grow differently in different places

Grade Level: 7

Subject: Red lettuce introduction

Objective: The purpose of this lesson is to introduce the concept of red lettuce and the fact that it may grow different in different places. Students will also begin to get used to touching plants.

Learning Outcomes:

Students are exposed to actual live plants in the form of seeds, environment for growing plants, getting seeds started, and the idea of recording information about plants to compare growth and understand differences. Students end the lesson having planted seeds and understand the initial garden center / windowsill environment. They may also share experiences where they have grown plants in the past, if applicable.

Activities:

Hook discussion (2 minutes):

Laura loves plants, and visited the garden center one weekend with her mom. Among the plants they were selling, Laura spotted some beautiful red lettuce seedlings (garden centers probably wouldn't sell live lettuce, just seeds. Maybe red Kale would be an option at the garden center, but this lesson focuses on lettuce). She decided that she wanted to grow some red lettuce herself at home, so she bought seeds from the garden center and took them home. Since it was still fairly cold outside, she planted them inside and kept them on her windowsill. As the seedlings sprouted and grew, however, Laura noticed that they were completely green! What would make these lettuce seedlings be green when the adult plants were red?

Discussion (5-10 minutes):

- Have students identify differences between the garden center and the window sill.
- Potential answers
 - Outside vs inside. Light is less inside. Warmer inside and colder outside. Windows vs no windows. Planted in large pots vs small seedling. Seedlings are young and garden center plants may be older.
- Students may want to write differences on the board.
- Have students formulate a **hypothesis** about what makes lettuce red. At this point, they will not have enough knowledge to make great guesses, but they should get used to making guesses before having enough knowledge.

Planting Seeds 15-20 minutes:

- Each student receives at least one cup and one jiffy pellet.
- Students must soak jiffy pellets in warm water until fully expanded.

- Students take 2-3 lettuce seeds and gently push them into the surface of the pellets, ensuring they are covered but not too deep.
- Label and date their cups.
- Decoration of cups is optional.
- Give students their log sheet so they can begin to take notes.

Background Info:

Materials needed:

Jiffy Pellets - <https://www.amazon.com/Count-Jiffy-Pellets-Seeds-Starting/dp/B000EHJN7K>

Plastic Cups 9oz - https://www.amazon.com/TashiBox-Disposable-Plastic-Tumblers-Crystal/dp/B0727PTKVH/ref=sr_1_3?ie=UTF8&qid=1543788183&sr=8-3&keywords=9-oz+plastic+cups

Red Lettuce Seeds – <http://www.Johnnyseeds.com/> any red variety like Teodore, Vulcan, red butter, red oakleaf, Cherokee, Rouxai, etc. These may also be purchased at local garden centers.

Name: _____

Lettuce Log Sheet

Directions: Record the date, if you have watered, the number of leaves on your plant, and its' width.

	Date	Water?	Number of Leaves + Size
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Directions: In each box, write the date and draw a small colored picture of your plant.
How red is your lettuce?

Lesson 2

Title: What do plants need to grow?

Grade Level: 7

Subject: Plant Growth Necessities

Objective: Students will understand

- The definition of a plant
- What plants need to grow
- How to water a plant
- The definition of hydroponics
- An introduction to vocabulary like photosynthesis, transpiration, evaporation, and precipitation

Learning Outcomes:

Answer question: what do plants need to grow? How do they grow?

Activities:

Water Cycle Activity (30 minutes): 7 graders should already have an understanding of the basics of the water cycle, but this time can be used to review this material. Have students draw a picture with the water cycle of the lettuce plant, both indoors and outdoors - Precipitation: when the plant is watered (rain or by hand). Transpiration/evaporation: plant takes up water and transpires through stomata (through roots, out leaves). Condensation: dew forms in the morning on plants outside, and forms clouds which in turn produce rain. Students should turn in drawings.

Framing activity: Showing water movement through plants. Using cut celery, cups of water, and food coloring, demonstrate how food-colored water is taken up through celery stalk and colors the stalk. Students prepare celery stalks and cups of colored water at the beginning of the lesson, and by the end of the lesson, color will begin to appear in the stalk. By the next day, leaves and veins will be very colored.

Small group discussion and presentation(15-20 minutes): Begin by asking students what plants need to grow: Answer is light, oxygen, water, carbon dioxide and nutrients. Write these answers on the board. If they miss any, provide them. Students will get into small groups and list several places where plants do NOT grow. They will identify what key components are missing to support plant life. Coming together as a class, they will share group by group one of their ideas that has not been picked yet. Together as a class, you could also identify where on the globe such places would be found.

Hydroponics tutorial(10-15 minutes.): Students will check on their lettuce cups while teacher talks about proper water levels for plants, and how to apply liquid fertilizer safely and appropriately. Materials needed for this are MiracleGro liquid fertilizer or other store bought liquid fertilizer.

Background Info:

Vocabulary

Hydroponics: the process of growing plants in sand, gravel, or liquid, with added nutrients but without soil.

Photosynthesis- The process by which plants convert light energy into chemical energy.

Transpiration – The evaporation of water from plants. Transpiration in leaves occurs through the stomata.

Evaporation – Change in matter to a less dense phase due to an increase in energy.

Precipitation – Any product of condensation of the water in the atmosphere that is deposited onto the earth's surface.

Celery

Stalk

Experiment

-

<https://www.acs.org/content/dam/acsorg/education/resources/k-8/science-activities/motionenergy/graphing/celery-soaks-it-up-science-for-kids.pdf>

For 9oz cups with a jiffy pellet sitting in the middle: Water should be checked daily. Levels should not fall below .5-1 inch of water in the cup. Fertilizer should be added following the directions on the product, though this is likely to be only once a week or less in the first week, the more often as the plants grow.

Lesson 3

Title: Answering our questions: the Scientific Method

Grade Level: 7

Subject: Scientific Method

Objectives:

- To be able to list the steps of the scientific method and apply them to a question
- Find a place to grow lettuce and form a hypothesis about size/redness of growth.
- Produce a list of research questions.

Activities:

Worksheet and discussion period (45 minutes)

- 1) Students should pair up and copy down the steps of the scientific method.
- 2) Students should be given an overview of the lettuce experiment as presented in lesson 1. Students should write down any observations or questions they have about the lettuce scenario, or plants in general that they are interested in. They can do this in pairs.
- 3) After a few minutes of discussion, join pairs together to make groups of four and have them share their observations and questions. Have them make a list of five or so questions they have. These could be questions like, “Is the outdoor lettuce cold?” Or “How does photosynthesis work?” or even “How long does it take lettuce to grow?”
- 4) Have each group share the questions and write them on the board.
- 5) Go through each question and decide what background information we would need to answer it. Ask students how they would try to answer the question. Sort questions by difficulty of answering.
- 6) Students should construct a hypothesis on the red leaf lettuce question based on these discussions and their knowledge of the lettuce experiment.
- 7) Discuss the lettuce experiment. Will the experimental design help to answer our questions and address our hypotheses? How could we make the experiment better?
 - Experimental design: Students grown 1-2 seedlings each, in 1-2 different areas of the classroom, collect data on growth and appearance, and compare those data to each other and the teacher lettuce.
 - Experimental strengths: lots of lettuce seedlings in different areas means lots of data

- Experimental weaknesses: no replicates (multiple seedlings in the same environment to see if results are the same), no real control over environment aside from what is provided to the school, the classroom isn't optimized for plant growth so our seedlings will likely end up being weak and small.
- Making the experiment better: having groups of seedlings in the same spot to get more data about that environment, better control of the air temperatures in the classroom, better control of the lighting, more lighting in general.

8) Students select their lettuce seedling growing spot.

Fill out the accompanying worksheet with relevant details. Apply steps of scientific method to the lettuce using worksheet. Form a hypothesis about lettuce placement.

Teacher should save the list of questions student have come up with during this lesson to be used in lesson 9, their research day.

Background Info:

Scientific Method

Steps: 1) Make and observation. 2) Form a question. 3) Form a hypothesis. 4) Conduct an experiment. 5) Analyze data and draw a conclusion.

Name: _____

Why isn't our lettuce red?

Worksheet 1

Directions: Following the steps of the scientific method, identify the parts of our lettuce problem and formulate a hypothesis.

Step 1: Identify the problem

Step 2: Ask a question

Step 3: Form a hypothesis

Step 4: Make a prediction

Step 5: Test the prediction

Step 6: Summarize conclusions

Possible answers

Step 1: Identify the problem

Our problem is that we want red lettuce but our lettuce at home is green, even though we bought the right seeds. We want to be able to grow red lettuce at home.

Step 2: Ask a question

Students should be able to ask the question “Why is red lettuce green when grown indoors?” Or something along those lines, as long as the question concerns plant growth and differences in different environments.

Step 3: Form a hypothesis

Students should make a guess about the answer to their question here.

Step 4: Make a prediction

Based on the experiment, students should make a prediction about how their lettuce will grow.

Step 5: Test the prediction

Students should say how this experiment and the data gathered will help them answer the question – by making observations about where lettuce is reddest or biggest, we can figure out how to grow healthy red lettuce at home.

Step 6: Conclusion

Students will reference their hypothesis and say that they will make a decision on if it was right or not. They can also talk about why they still don’t know, and what else they will need to answer the question.

Lesson 3 Evaluation

Title: Quiz and picture time

Grade Level: 7

Subject: Water Cycle, Scientific Method

Objective:

Assess student understanding of scientific method lesson

Assess student ability for limited application of scientific method with a story problem and short response.

Instruct students on visual observation and recording

Learning Outcomes:

Completed quiz

Completed worksheet with one drawing, leaf count, and measurement

Activities:

Quiz worksheet – 3 to 5 questions on vocabulary and basic knowledge of water cycle.
1 application question on scientific method

Background Info:

Not much required for this. Teacher must understand the correct answers to the questions asked in quiz, which can be written to their discretion. Previous lecture materials can be used as background info.

This is the first time the students will be using their log sheets for recording information. Students begin by recording the date. They then will record if their plant has water and if they need to water it. This could be yes/no, or water level. They will then count the total number of leaves that their seedling has, being careful not to damage them. To accomplish this, students may pick up their Jiffy pellets from their cups and turn the seedling to see, or they can remain in the cups. Then, students must measure their seedlings using a ruler. They should be concerned with how wide the plant is, from leaf tip to leaf tip, at the widest point. Students may use millimeters, centimeters, or inches.

Scientific method quiz generator:
[https://www.helpsteaching.com/questions/Scientific Method/Grade 6](https://www.helpsteaching.com/questions/Scientific_Method/Grade_6)

Some story problems:
[https://drrossymathandscience.weebly.com/uploads/1/6/6/9/16699960/scientific method story worksheet.pdf](https://drrossymathandscience.weebly.com/uploads/1/6/6/9/16699960/scientific_method_story_worksheet.pdf)

Lesson 4

Title: How do plants use light?

Grade Level: 7

Subject: Photosynthesis, light

Objectives:

- Understand photosynthesis in general terms (steps, materials required, maybe the formula)
- How to read simple chemical equations

Learning Outcomes:

- Be able to talk about how plants use light for energy in general terms
- Be able to identify organisms that use photosynthesis
- Be able to name plant-based cell structures

Activities:

Intro lecture (5-10 minutes): Short talk to define vocabulary, show the process of photosynthesis, or use of reading resources for short reading time. This could also have been assigned as homework.

Photosynthesis activities (online) (30+ minutes):

Using the following activity, draw a diagram of a leaf actively photosynthesizing, labeling all of the components used and where reactions happen. One for day, and one for night. https://authoring.concord.org/activities/1008/single_page/08901e16-9287-4d75-bc2e-647c05c07ed3

Alternatively, students can answer the questions given on the site by writing them out.

Discussion (10-15 minutes): Define and list photosynthetic organisms (green things), list on board or look up pictures online to share. Students could form small groups and either list several photosynthetic organisms (species or group) or look for a couple of interesting facts about a single organism. Can't use lettuce! The internet can be a resource, or books in the classroom, or personal experiences.

Background Info:

Photosynthesis worksheet from BTMS, Fort Mills:
http://btms.fortmillschools.org/UserFiles/Servers/Server_57118/File/photosynthesis_worksheet_HW.pdf

Plants and other photosynthetic organisms are the only organisms that can take light energy and convert it directly to chemical energy. For plants, this is how they get most of their energy, much like animals must eat to stay alive. Plants are considered photoautotrophs, which means their primary source of energy is light, while animals would be considered heterotrophs, or organisms that have to obtain their nutrition from outside of themselves. Light energy is

stored by proteins called reaction centers. In plants, these are held in the chloroplasts, and the reaction centers are green chlorophyll pigments.

Plant cells contain chlorophyll, which is a special pigment found in plants and other photosynthetic organisms, that allows photosynthesis to take place. Many chlorophyll complexes are found inside chloroplasts, which is the cellular structure holding them together.

As sunlight hits a leaf, chlorophyll within the plant leaves receives the light energy and converts it to chemical energy by breaking down carbon dioxide molecules and water molecules to produce sugars and oxygen. These sugars are stored within the plant for various uses, and the oxygen is released into the atmosphere. Photosynthesis is the reason we have oxygen to breathe!

Vocabulary –

Photosynthesis - the process by which green plants and some other organisms use sunlight to synthesize foods from carbon dioxide and water. Photosynthesis in plants generally involves the green pigment chlorophyll and generates oxygen as a byproduct.

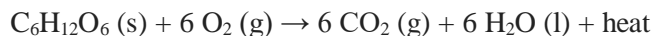
6CO_2 (Carbon Dioxide) + $6\text{H}_2\text{O}$ (Water) $\xrightarrow{\text{Light}}$ $\text{C}_6\text{H}_{12}\text{O}_6$ (Sugar) + 6O_2 (oxygen)

Chlorophyll - a green pigment, present in all green plants and in cyanobacteria, responsible for the absorption of light to provide energy for photosynthesis. Its molecule contains a magnesium atom held in a porphyrin ring.

Chloroplasts – a plastid containing chlorophyll and other pigments, occurring in plants and algae that carry out photosynthesis.

carbon dioxide – a gaseous compound containing one carbon and two oxygen atoms. Carbon dioxide is breathed out by animals as a result of cellular respiration.

Cellular respiration – a process that occurs in cells that converts chemical energy from sugars and oxygen into carbon dioxide, water, and heat. This reaction may be thought of in loose terms as the opposite of photosynthesis.



Glucose - a simple sugar that is an important energy source in living organisms and is a component of many carbohydrates.

wavelength,

photoautotroph – an organism that derives its energy for food synthesis from light and is capable of using carbon dioxide as its principal source of carbon.

autotroph – an organism that is able to form nutritional organic substances from simple inorganic substances such as carbon dioxide.

Heterotroph – an organism deriving its nutritional requirements from complex organic substances.

Photosynthetic organisms -> Algae, plants, cyanobacteria.

Lesson 5

Title: Quiz and hypothesis discussion

Grade Level: 7

Subject: Light, photosynthesis, scientific method

Objective:

- To assess student understanding of lessons
- To log data for class experiment

Learning Outcomes:

- Gaps in understanding of scientific method identified
- Application of scientific method to reinforce previous learning
- Students will have shared in small groups and in front of the class

Activities:

Quiz (15-20 minutes) – application oriented quiz, some photosynthesis vocabulary. Students should be given another story problem and be able to come up with a hypothesis and an experiment to test in a short answer? Maybe draw a diagram of the process of photosynthesis including sun, water, plant, carbon dioxide, and sugar, and label where each component is in the process. This is at the teachers discretion.

Think, pair, share(20+ minutes) – Hand back the scientific method worksheet. Students group up in groups of 2-3. Objective is to discuss the hypothesis that they developed. Amongst themselves, they share their hypotheses. If they all have a similar hypothesis, they present only one hypothesis to the class and write it on the board. If their hypotheses are different, they choose two and write them on the board. OR we go around the room and each group shares one hypothesis and the reasons why they believe their hypothesis and where they are growing their lettuce. 5-10 minutes to talk in group, 5-10 minutes to share

Lettuce check – students check water levels of their lettuce cups and make observations on their log sheets. Drawing of lettuce, leaf count, measure size. Class lettuce checks for data should occur at least once a week. Students should monitor their lettuce for water needs on a daily basis. (See log sheet, lesson 1) 5-10 minutes

Background Info:

All necessary background info contained in the photosynthesis and scientific method lesson

Lesson 6

Title: Data and comparisons

Grade Level: 7

Subject: Making observations and comparing them

Objective:

- Practice data collection
- Practice putting data in order
- Practice analyzing data as a group

Learning Outcomes:

- Rank plants in order of size
- Rank plants in order of redness
- Rank plants in order of number of leaves (maybe)
- Analysis of best growing spots
- Formulation of a new hypothesis

Activities:

Lettuce maintenance and data collection. Students will water their lettuce, count leaves, measure size. 5-10 minutes

Ranking exercises. First, using the data they have collected, students should line up in order of largest to smallest lettuce heads. Give them each a “rank”, with 1 being largest and the last number being the smallest. Ask students to point out where in the room their lettuce is growing. Why do they think their lettuce is bigger or smaller than another spot? Ask them to name at least one environmental condition that could be causing this. Ask them what their initial hypothesis was for size. Is their lettuce growing like they expected it to? These questions will be responded to on a worksheet.

Ranking exercises. Second, as students to compare their pictures for “redness”. This may be more difficult and subjective. Students may want to hold their actual lettuce during either of these exercises. Students should record their number in either ranking. Is their lettuce growing as expected? Students will record responses in their worksheet.

Ranking exercises. The teacher should bring out the teacher lettuce as well to rank it for both size and redness in both rankings. 40-50 minutes

Background Info:

None necessary.

Name: _____

Date: _____

Ranking Classroom Lettuce

Directions: Fill out each question as your teacher takes you through the ranking exercises.

Exercise 1: Lettuce size comparison

As a class, retrieve your lettuce from its' growing place. Compare your lettuce to your classmate's and arrange yourselves in a line, with largest lettuce on one end and smallest lettuce on the other. Some lettuce may be the same size! Number yourselves starting from 1 for largest.

Your lettuce number: _____

Total class number: _____

Were there lettuce the same size as yours? _____

What was the biggest lettuce measurement? _____

What was the smallest lettuce measurement? _____

How big was the teacher lettuce? _____

Exercise 2: Lettuce color comparison

Now that you've ranked lettuce size, let's rank lettuce color. Your lettuce should be red, but red lettuce can range from completely red to completely green. As a class, compare HOW red your lettuce is. Estimate the amount of red contained in your lettuce leaves and arrange yourselves in a line , with the reddest lettuce being 1.

Your lettuce number: _____

Total class number: _____

Were there lettuce the same color as yours? _____

How red was the teacher lettuce? _____

Name one environmental condition that could cause your lettuce to be different than others:

Is your lettuce growing according to your predictions? How? Why or why not?

Lesson 7

Title: Investigating Light Sources

Grade Level: 7

Subject: Color of light

Learning Outcomes:

- Understand connection between color and wavelength of light
- Be able to generally identify the presence of different colors of light in different light sources
- Connect these colors of light to photosynthesis and the blue / red LEDs being used to grow the teacher lettuce (See lettuce experiment setup)
- Identify what colors of light student seedlings are receiving.

Activities:

Spectroscope activity: looking at different light sources and viewing the spectrum visible in the visor. This could be done with the spectroscopes that the children constructed. Children should draw spectrums on paper to record the colors present in different light sources. Children should look at the combination of red and blue light generated by the shoebox lettuce lights that appears pink, and will see that the spectroscope separates the light into red and blue once more. Source:

Making a cheap spectroscope: <https://www.livescience.com/41548-spectroscopy-science-fair-project.html>

Second spectroscope option: This is a smaller one: <https://buggyandbuddy.com/homemade-spectroscope/>

Background Info:

Plants use photosynthesis to grow and can use any light with wavelengths between 400 – 700 nanometers. This is called “photosynthetically active radiation”. Plants are able to use red light most efficiently for photosynthesis, with blue light being the next best. In nature, plants evolved using our sun to perform photosynthesis, which is a “broad spectrum” light. This means that, were we to look at the sun with a spectroscope, a full spectrum of all colors would be visible. With indoor agriculture, plants grow under a couple different kinds of light. Growers can either use “broad spectrum lights”, such as fluorescent or high-pressure sodium lights, or they can use LEDs, which have very specific colors or spectrums designed for plant growth.

Visible light has a wavelength that ranges from approximately 400-700 nanometers. A spectroscope separates visible light into the wavelengths that are present by passing it through a diffraction grating. The different energy levels represented by the different colors of light cause them to bend differently when passing through different materials or around edges. See live science activity for more detailed information.

How is it that when we look at white lights, we see white and not all the colors at once? Our eyes can be fooled into seeing a single color due to the way the color cones behind

our eyes work. Just like with the LEDs. When you turn on only blue, we only see blue. When we turn on only red, we only see red. But with both lights on at once, we see pink!

When applying the spectroscope to the lettuce experiment, students should begin by examining light sources near to their seedling growth area. While standing near their seedling, they should identify the nearest or brightest light source. This light source should be examined through the spectroscope. What colors do they see that are present in the spectroscope? How does this compare to the LEDs in the shoebox? How about the sun? Is the light source even bright enough for them to distinguish colors? Their nearest or brightest light sources may be too dim for them to tell anything – this is also an important result! Students can get a feeling for how bright their lights are, what colors their lettuce are exposed to, and compare these spectra with the class. There should be some connections made to both size of the lettuce seedlings and color of the seedlings. Seedlings getting more or brighter light will likely be larger than seedlings with dimmer light. Seedlings being exposed to more blue light will likely be more red than seedlings with less blue light. Students should begin to try drawing these conclusions by looking at the data.

Name: _____

Spectroscope Spectra

Different light sources contain different colors! Using your spectroscope, seek out 3 different light sources and record their spectra. Examples of light sources could be a candle, a fluorescent light versus an incandescent bulb, an LED light, the sun reflected off a white sheet of paper. If you are unsure of what type of light your source is, ask your teacher!

For your 4th light source, stand by your lettuce seedling and determine which light source is the brightest in this location. Record the spectra that your lettuce seedling is exposed to.

Light Source	Spectra (Color it in!)

Lesson 8

Title: How do we do research?

Grade Level: 7

Subject: Textual research assignment

Objective:

Students will make an effort to do background research in and out of the classroom to answer questions they may have had.

Learning Outcomes:

- Gain experience with internet and library research.
- Gain experience with report writing.

Activities:

Library day (1 hour): In the library, or with computers, take students through how to research answers to any type of question. This part of the procedure will be dependent upon resources available.

Students will pick a question to research and respond to a writing prompt. This exercise should include a paragraph or two of writing, and location of one or two sources for their information. As an introduction to this lesson, the questions students came up with during lesson 3 should be brought up again, as well as an initial brainstorming session to build upon them. After the brainstorming session to generate a list of questions, students can select one they are particularly interested in and use library resources to research an answer to their question. They may find the answer to “why is my red lettuce green?” during this period; this would be an acceptable result. Exemplary responses will address the question, answer it, and apply that information to their hypothesis.

Students should turn in this assignment during the next class period to allow for extra at-home research and writing time if necessary. Assignment length and number of sources should be adjusted dependent upon how much emphasis the teacher would like to place upon written research skills.

Background Information:

Citation how-to's: https://owl.purdue.edu/owl/research_and_citation/resources.html

Name: _____

Research Project

Directions: Choose a question from the class brainstorming session. Use library or online resources to locate two sources that answer this question. Record the citation for each source on this sheet of paper. On a separate sheet of paper (typed or handwritten), attempt to answer your selected question in one or two paragraphs. How does this question relate to your lettuce hypothesis?

Lesson 9

Title: Data and comparisons

Grade Level:7

Subject: Making observations over time and comparing them

Objective:

- Practice data collection
- Practice putting data in order
- Practice analyzing data as a group

Learning Outcomes:

- Rank plants in order of size
- Rank plants in order of redness
- Rank plants in order of number of leaves (maybe)
- Analysis of best growing spots
- Formulation of a new hypothesis

Activities:

Lettuce maintenance and data collection. Students will water their lettuce, count leaves, measure size. 5-10 minutes

Ranking exercises. First, using the data they have collected, students should line up in order of largest to smallest lettuce heads. Give them each a “rank”, with 1 being largest and the last number being the smallest. Ask students to point out where in the room their lettuce is growing. Why do they think their lettuce is bigger or smaller than another spot? Ask them to name at least one environmental condition that could be causing this. Ask them what their initial hypothesis was for size. Is their lettuce growing like they expected it to? These questions could be responded to on a worksheet.

Ranking exercises. Second, as students to compare their pictures for “redness”. This may be more difficult and subjective. Students may want to hold their actual lettuce during either of these exercises. Students should record their number in either ranking. Is their lettuce growing as expected? Students will record responses in their worksheet.

Ranking exercises. The teacher should bring out the teacher lettuce as well to rank it for both size and redness in both rankings. 40-50 minutes

Background Info:

This is the second ranking exercise performed. The results from the previous ranking exercise should be compared to this ranking exercise.

Name: _____

Date: _____

Ranking Classroom Lettuce

Directions: Fill out each question as your teacher takes you through the ranking exercises.

Exercise 1: Lettuce size comparison

As a class, retrieve your lettuce from its' growing place. Compare your lettuce to your classmate's and arrange yourselves in a line, with largest lettuce on one end and smallest lettuce on the other. Some lettuce may be the same size! Number yourselves starting from 1 for largest.

Your lettuce number: _____

Did your number change?: _____

Were there lettuce the same size as yours? _____

What was the biggest lettuce measurement? _____

What was the smallest lettuce measurement? _____

How big was the teacher lettuce? _____

Exercise 2: Lettuce color comparison

Now that you've ranked lettuce size, let's rank lettuce color. Your lettuce should be red, but red lettuce can range from completely red to completely green. As a class, compare HOW red your lettuce is. Estimate the amount of red contained in your lettuce leaves and arrange yourselves in a line , with the reddest lettuce being 1.

Your lettuce number: _____

Did your number change?: _____

Were there lettuce the same color as yours? _____

How red was the teacher lettuce? _____

Is your lettuce growing according to your predictions? How? Why or why not?

Lesson 10

Title: Current events in horticulture research

Grade Level: 7

Subject: examples of current research

Objective: To inspire interest in a career in horticulture

Learning Outcomes:

Students will see some examples of where studying horticulture will take them

Activities:

This lesson can take two different forms.

Option 1) Video day with accompanying worksheet. A series of videos is shared about researchers in different projects involving horticulture topics

Space agriculture – The Lunar Greenhouse, NASA’s Big Idea Challenge

<https://cals.arizona.edu/earthlight/videos>

Improving Field Crops – Genetic modification of crops, conventional breeding, orchard and field management

<https://www.youtube.com/watch?v=QJK4sN91ekE>

<https://youtu.be/6l47wXPHOEA>

Humor - <https://www.youtube.com/watch?v=zY-UPHLJ31A>

Plant Propagation Specialists – Grafting operations, plant tissue culture

<https://www.youtube.com/watch?v=zhgsPkeZEBk>

https://www.youtube.com/watch?v=jzLrS_7pQXc

Curation of Botanical Gardens, Plant Hunters, Herbarium –

<https://www.youtube.com/watch?v=Sp4k0Ux0H8M>

Turf Grass Specialists – Cemeteries, golf courses.

<https://www.youtube.com/watch?v=tzX5P5FpYdk>

<https://www.youtube.com/watch?v=UnzYq1d0Zjw>

Extension Agents –

<https://www.youtube.com/watch?v=0C9PYINhQOU>

<https://youtu.be/XGCz6wffWDg>

Lettuce Field Production –

https://www.youtube.com/watch?v=KTcKy_6TKk0

Option 2) Visiting a nearby horticulture-oriented entity.

College – Seeing real-life, current examples of horticulture research either in a lab or in a green house or both.

Nursery – Some nurseries have trial gardens and active research.

Botanic Garden – Some botanic gardens have trial gardens or active research areas working with nearby schools.

Students must complete a work sheet that identifies the main purpose of the research they are seeing, plants involved, how is lighting involved in this study? For outdoor operations, they can talk about the sun and day period and seasonality.

Teacher can to choose to do one or the other or both of these activities. Teacher should pick and choose which subset of videos will be most relevant to their particular class, and should feel free to add in their own if they have any of particular interest not listed.

Background Info:

Video links included, though video selections can be edited as needed by teachers. If the second activity is selected, a custom worksheet should be developed based upon the operation being visited.

Worksheet activity

Name: _____

Plant Science in the Field

Directions: For each video, take notes and write up a short summary to answer the following questions.

Where did the video take place?

What activities did you see happening in the video?

Was there a job title mentioned in the video?

How is plant science involved in these activities?

Lesson 11

Title: What do you want to be when you grow up?

Grade Level: 7

Subject: Career and personality testing (OPTIONAL)

Objective: Gain an idea of careers that may suit students' interests

Learning Outcomes: Generate a list of potential careers; understand what type of education will help them get there; gain a better understanding of their interests

Activities:

- Personality tests
- Career test
- Career search
- Select from the provided activities from FFA and careerplanner.org. These activities may be completed over the course of 1-2 days.

Name: _____

What do I want to be when I grow up?

Directions: Go to educationplaner.org. Under Career Planning, open Find Careers and complete the Career Clusters Activity. Once completed, select two careers in your Interest Areas and record the following information. If no information is available, write “Not Applicable”.

Occupation 1: _____

Wages: _____

Education Needed: _____

Responsibilities:

Skills needed (2-3):

Under the “Find Training” option, write one option for each, including program name.

<1 year options? : _____

2 year options? : _____

4 year options? : _____

Why do you like this career?

Occupation 2 : _____

Wages: _____

Education Needed: _____

Responsibilities:

Skills needed (2-3):

Under the “Find Training” option, write one option for each, including program name.

<1 year options? : _____

2 year options? : _____

4 year options? : _____

Why do you like this career?

Lesson Plan

Around the World

Created: 04/2017 by the National FFA Organization

STUDENT LEARNING OBJECTIVES After completing these activities students will...

1. Compare and contrast agricultural careers around the world. 2. Identify cultural differences in countries around the world. 3. Explain varying qualifications for careers around the world compared to the U.S.

TIME REQUIRED: 45 minutes

RESOURCES:

1. FFA.org 2. Website – National Geographic: <http://travel.nationalgeographic.com/travel/countries/>

EQUIPMENT AND SUPPLIES NEEDED:

1. A copy of the “Around the World” worksheet for each student. 2. Internet access for the website for each student.

THIS QUICK LESSON PLAN WOULD WORK WELL AS:

1. Part of a unit about agriculture innovators and/or companies. 2. Part of a unit about agriculture careers. 3. Part of a unit about agriculture advances. 4. Part of a unit about agricultural business or marketing.

THESE ACTIVITIES ARE ALIGNED TO THE FOLLOWING STANDARDS:

FFA Precept

- FFA.PL-A.Action: Assume responsibility and take the necessary steps to achieve the desired results, no matter what the goal or task at hand.
- FFA.PG-I.Professional Growth: Assume responsibility for attaining and improving upon the skills needed for career success.
- FFA.PG-J.Mental Growth: Embrace cognitive and intellectual development relative to reasoning, thinking and coping.
- FFA.CS-M.Communication: Effectively interact with others in personal and professional settings. AFNR Cluster Skills
- CS.01. Analyze how issues, trends, technologies and public policies impact systems in the Agriculture, Food & Natural Resources Career Cluster. Common Core- Writing
- CCSS.ELA-LITERACY.W.9-10.1 Write arguments to support claims in an analysis of substantive topics or texts, using valid reasoning and relevant and sufficient evidence.

- CCSS.ELA-LITERACY.W.9-10.2 Write informative/explanatory texts to examine and convey complex ideas, concepts, and information clearly and accurately through the effective selection, organization, and analysis of content.
- CCSS.ELA-LITERACY.W.9-10.4 Produce clear and coherent writing the development, organization, and style are appropriate to task, purpose, and audience. Common Core- Speaking and Listening
- CCSS.ELA-LITERACY.SL.9-10.1 Initiate and participate effectively in a range of collaborative discussions (one-on-one, in groups, and teacher-led) with diverse partners on grades 9-10 topics, texts, and issues, building on others' ideas and expressing their own clearly and persuasively.
- CCSS.ELA-LITERACY.SL.9-10.4 Present information, findings, and supporting evidence clearly, concisely, and logically such that listeners can follow the line of reasoning and the organization, development, substance, and style are appropriate to purpose, audience, and task. Common Core- Language
- CCSS.ELA-LITERACY.L.9-10.6 Acquire and use accurately general academic and domain-specific words and phrases, sufficient for reading, writing, speaking, and listening at the college and career readiness level; demonstrate independence in gathering vocabulary knowledge when considering a word or phrase important to comprehension or expression.

NAME:

Around the World

DIRECTIONS:

5. With the help of the National Geographic website (<http://travel.nationalgeographic.com/travel/countries/>), answer the questions below.

1. *On the map below, label five countries that you would like to explore further.*



Aligned to the following standards: FFA.PL-A; FFA.PG-I; FFA.PG-J; FFA.CS-M; CS.01; CCSS.W.9-10.1; CCSS.W.9-10.2; CCSS.W.9-10.4; CCSS.SL.9-10.1; CCSS.SL.9-10.4; CCSS.L.9-10.6; CCSS.MP3; CCSS.MP6; CCSS.MP7; CRP.02; CRP.04; CRP.05; CRP.06; CRP.08

2. *Identify one agriculture career that interests you. Describe that career below.*

3. For each of the countries that you identified in the map above, explore your chosen agriculture career in each of those countries. Describe how it

may differ from the United States. Capture your notes in the space below.

Country 1:

Country 2:

Country 3:

Country 4:

Country 5:

Lesson Plan

College Search

Created: 04/2018 by the National FFA Organization

STUDENT LEARNING OBJECTIVES: After completing these activities, students will...

1. Discuss the pros and cons of attending college. 2. Examine information about five different colleges. 3. Determine the diversity of agriculture colleges within the United States.

TIME REQUIRED: 45 minutes

RESOURCES:

1. FFA.org 2. Article — “Is College Right for Me? The Pros and Cons of College,” <https://fremont.edu/is-college-right-for-me-the->

[pros-and-cons-of-college/](https://fremont.edu/is-college-right-for-me-the-pros-and-cons-of-college/) 3. AgExplorer — <https://www.AgExplorer.com/>

EQUIPMENT AND SUPPLIES NEEDED:

4. A copy of the “College Search” worksheet for each student. 5. Internet access to read the online article or print it ahead of time.

THIS QUICK LESSON PLAN WOULD WORK WELL AS:

1. A portion of a careers unit (for any career focus area). 2. An activity in an SAE unit.

THESE ACTIVITIES ARE ALIGNED TO THE FOLLOWING STANDARDS:

AFNR Performance Element

- CS.05. Describe career opportunities and means to achieve those opportunities in each of the Agriculture, Food & Natural Resources career pathways. FFA Precept
- FFA.PL-C.Vision: Visualize the future and how to get there.
- FFA.PL-E.Awareness: Understand personal vision, mission and goals.
- FFA.PL-F.Continuous Improvement: Accept responsibility for learning and personal growth.
- FFA.PG-I.Professional Growth: Assume responsibility for attaining and improving upon the skills needed for career success.
- FFA.PG-J.Mental Growth: Embrace cognitive and intellectual development relative to reasoning, thinking and coping.
- FFA.CS-M.Communication: Effectively interact with others in personal and professional settings.

- FFA.CS-N.Decision Making: Analyze a situation and execute an appropriate course of action. Common Career Technical Core

- AG5 Describe career opportunities and means to achieve those opportunities in each of the Agriculture, Food & Natural Resources Career Pathways. NASDCTEc

- AGC09.02 Select, research and examine critical aspects of career opportunities in one or more AFNR career pathways in order to gain an understanding of the breadth of occupations within this cluster. Common Core- Reading: Informational Text

- CCSS.ELA-Literacy.RI.9-10.4 Determine the meaning of words and phrases as they are used in a text, including figurative, connotative, and technical meanings; analyze the cumulative impact of specific word choices on meaning and tone (e.g., how the language of a court opinion differs from that of a newspaper)." Common Core- Writing

- CCSS.ELA-Literacy.W.9-10.2 Write informative/explanatory texts to examine and convey complex ideas, concepts, and information clearly and accurately through the effective selection, organization, and analysis of content." Common Core- Speaking and Listening

- CCSS.ELA-Literacy.SL.9-10.1 Initiate and participate effectively in a range of collaborative discussions (one-on-one, in groups, and teacher-led) with diverse partners on grades 9-10 topics, texts, and issues, building on others' ideas

Lesson 12

Title: Data and comparisons

Grade Level: 6-8

Subject: Making observations over time and comparing them

Objective:

- Practice data collection
- Practice putting data in order
- Practice analyzing data as a group
- Compare data to previous class data collection activity (lesson 9)

Learning Outcomes:

- Rank plants in order of size
- Rank plants in order of redness
- Rank plants in order of number of leaves (maybe)
- Analysis of best growing spots
- Formulation of a new hypothesis

Activities:

Lettuce maintenance and data collection. Students will water their lettuce, count leaves, measure size. 5-10 minutes

Ranking exercises. First, using the data they have collected, students should line up in order of largest to smallest lettuce heads. Give them each a “rank”, with 1 being largest and the last number being the smallest. Ask students to point out where in the room their lettuce is growing. Why do they think their lettuce is bigger or smaller than another spot? Ask them to name at least one environmental condition that could be causing this. Ask them what their initial hypothesis was for size. Is their lettuce growing like they expected it to? These questions could be responded to on a worksheet.

Ranking exercises. Second, as students to compare their pictures for “redness”. This may be more difficult and subjective. Students may want to hold their actual lettuce during either of these exercises. Students should record their number in either ranking. Is their lettuce growing as expected? Students will record responses in their worksheet.

Ranking exercises. The teacher should bring out the teacher lettuce as well to rank it for both size and redness in both rankings. 40-50 minutes

Switch the teacher lettuce treatments. Make sure the students have gotten a drawing or pictures of the lettuce growing in the red/blue boxes. Make a show of switching the red lit lettuce to blue, and vice versa. Ask the students to propose a hypothesis for what will happen. Will something happen to one? to both? to neither? Have them write down their hypotheses on a

sheet of paper and turn it in. In two days, before the salad party, return the student's hypotheses to them when evaluating the effects of the lighting treatments. Who was totally right? Who was partially right? Who was completely wrong? It must somehow be emphasized that being wrong doesn't mean they failed, as a wrong hypothesis just means that we must adjust our hypothesis to include new data. Everyone gets 100% as long as they made a guess. Compare these hypotheses to their original hypotheses for how their lettuce would grow and why the original lettuce was green and not red in the girl's windowsill.

Lesson 13

Title: Party Time!

Grade Level: 7

Subject: Harvest safety

Objective:

- Learn food safety principles
- Answer the original question – why was the indoor lettuce green and the outdoor lettuce red?
- Identify best place in the classroom for growing large lettuce? red lettuce?

Learning Outcomes:

- Enjoy delicious salad with lots of toppings but also the student grown lettuce
- Put hypotheses into context, to get kids engaged in discussion

Activities:

Begin with final data collection on their log sheets. Maybe take pictures of all the kids holding their lettuce. After a couple of ranking sessions, the kids should have a good idea of which lettuce did the best. 5-10 minutes.

Harvest. Gather materials (scissors, dish soap, maybe bleach). Sanitize hands and scissors. Wear gloves (optional but maybe a good idea). Cut head of lettuce right above the Jiffy pellet. Rinse each head of lettuce under running water. Shake gently. Cut the base of the lettuce to release all leaves. Place rinsed and cut leaves into salad spinner. Fill as needed with store bought salad mix. Examine the store bought mix. Is there any red lettuce in it? Maybe purchase a baby leaf mix, as their lettuce heads will be close to baby leaf size. Allow kids to take a turn on the salad spinner if they want. Set up salad bar (and any other food you would like to include). 10-20 minutes?

Eat! 10 minutes?

Discussions questions: who had the largest lettuce? Where was the best place in the room to grow lettuce? Why do we think that was? Who had the reddest lettuce? How does this compare to the original question of red lettuce outside and green lettuce inside? What happened to the teacher lettuce when we switched the lights? This discussion maybe can take place during eating or afterwards. A worksheet that they can fill out while/after they eat, and students can answer after they have written down their answers. 10-20 minutes?

Name: _____

Date: _____

Ranking Classroom Lettuce

Directions: Fill out each question as your teacher takes you through the ranking exercises.

Exercise 1: Lettuce size comparison

As a class, retrieve your lettuce from its' growing place. Compare your lettuce to your classmate's and arrange yourselves in a line, with largest lettuce on one end and smallest lettuce on the other. Some lettuce may be the same size! Number yourselves starting from 1 for largest.

Your final lettuce number: _____

Where and what was the biggest lettuce measurement?

Where and what was the smallest lettuce measurement?

How big was the red and blue teacher lettuce?

Exercise 2: Lettuce color comparison

Now that you've ranked lettuce size, let's rank lettuce color. Your lettuce should be red, but red lettuce can range from completely red to completely green. As a class, compare HOW red your lettuce is. Estimate the amount of red contained in your lettuce leaves and arrange yourselves in a line, with the reddest lettuce being 1.

Your final lettuce number: _____

Where was the reddest lettuce?

What happened to the teacher lettuce when we switched the lights?

What was your hypothesis? How has it changed? Why or why not?

Appendix II: Statistical Methods and Code

Method Definitions

lmerTest

Provides p-values in type I, II or III anova and summary tables for lmer model fits (cf. lme4) via Satterthwaite's degrees of freedom method. A Kenward-Roger method is also available via the pbkrtest package. Model selection methods include step, drop1 and anova-like tables for random effects (ranova). Methods for Least-Square means (LS-means) and tests of linear contrasts of fixed effects are also available.

<https://cran.r-project.org/web/packages/lmerTest/index.html>

lme4

Fit linear and generalized linear mixed-effects models. The models and their components are represented using S4 classes and methods. The core computational algorithms are implemented using the 'Eigen' C++ library for numerical linear algebra and 'RcppEigen' "glue".

<https://cran.r-project.org/web/packages/lme4/index.html>

emmeans

Obtain estimated marginal means (EMMs) for many linear, generalized linear, and mixed models. Compute contrasts or linear functions of EMMs, trends, and comparisons of slopes. Plots and compact letter displays. Least-squares means are discussed, and the term "estimated marginal means" is suggested, in Searle, Speed, and Milliken (1980) Population marginal means in the linear model: An alternative to least squares means, The American Statistician 34(4), 216-221 <[doi:10.1080/00031305.1980.10483031](https://doi.org/10.1080/00031305.1980.10483031)>.

<https://cran.r-project.org/web/packages/emmeans/index.html>

cld

This function uses the Piepho (2004) algorithm (as implemented in the multcompView package) to generate a compact letter display of all pairwise comparisons of least-squares means. The function obtains (possibly adjusted) *P* values for all pairwise comparisons of means, using the [contrast](#) function with `method = "pairwise"`. When a *P* value exceeds `alpha`, then the two means have at least one letter in common.

<https://www.rdocumentation.org/packages/lsmeans/versions/2.27-62/topics/cld>

Tomatoes

Obtaining averages

```
emmeans(lmer(Weight~Treatment + (1|Group), data = tHarvests), pairwise ~ Treatment, response = "type")
```

Tukey's Groupings with Letters

```
install.packages("multcompView")  
cld(weightMeans)
```

Harvest Weights

```
hWeight<- lmer(Weight ~ Treatment + Experiment + Chamber + (1|Group/Plant), data=tHarvests)
```

```
weightMeans <- emmeans(hWeight, pairwise ~ Treatment, response = "type")
```

Fruit count

```
fCount <- glmer(Number ~ Treatment + Experiment + Chamber + (1|Group/Plant/Harvest),  
family=poisson, data=tHarvests)
```

```
countMeans <- emmeans(fCount, pairwise ~ Treatment, response="type")
```

Average Size

```
avgSize <- lmer(AverageWt ~ Treatment + (1|Group/Plant) + Chamber + Experiment, data =  
tHarvests)
```

```
sizeMeans <- emmeans(avgSize, pairwise ~ Treatment, response = "type")
```

Brix

```
hBrix <- lmer(BRIX ~ Treatment + Experiment + Chamber + (1|Group/Plant), data = tHarvests)
```

```
brixMeans <- emmeans(hBrix, pairwise ~ Treatment, response = "type")
```

Seedling Height

```
sHeight <- lmer(Height ~ Chamber + Experiment + Treatment + TimePoint +  
TimePoint*Treatment + (1|Group), data=tHeight)
```

```
heightMeans <- emmeans(sHeight, pairwise ~ Treatment|TimePoint, response = "type")
```

Fruits

```
fruitsFlowers$Age <- as.factor(fruitsFlowers$Age)
```

```
> devFruit <- glm(Fruits ~ Treatment + Age + Age*Treatment, family=poisson,  
data=fruitsFlowers)
```

```
> devFrMeans <- emmeans(devFruit, pairwise ~ Treatment|Age, response = "type")
```

```
> devFrMeans
```

Flowers

```
> devFlowers <- glm(Flowers ~ Treatment + Age + Age*Treatment, family=poisson,  
data=fruitsFlowers)
```

```
> devFlMeans <- emmeans(devFlowers, pairwise ~ Treatment|Age, response = "type")
```

Ascorbic Acid Content

```
> acidCont <- lm(AscAcid ~ Treatment + Date, data = ascAcid)
```

```
> emmeans(acidCont, pairwise ~ Treatment, response = "type")
```

Lettuce

Weight

```
> lWeight <- lmer(Weight ~ Lights + Variety + (1|Experiment) +
(1|Experiment:Lights:Variety:Block) + Lights*Variety, data=phase2)
> lWeightMeans <- emmeans(lWeight, pairwise ~ Lights|Variety, response="type")
```

Height

```
lHeight <- lmer(Height ~ Lights + Variety + (1|Lights:Variety:Block) + Lights*Variety,
data=lSize)
lHeightMeans <- as.data.frame(emmeans(lHeight, pairwise ~ Lights|Variety, response="type"))
```

Diameter

```
> lWidth <- lmer(Width ~ Lights + Variety + (1|Lights:Variety:Block) + Lights*Variety,
data=lSize)
> lWidthMeans <- as.data.frame(emmeans(lWidth, pairwise ~ Lights|Variety, response="type"))

> lBrix <- lmer(BRIX ~ Light + Variety + Light*Variety + (1|Light:Variety:Block), data=BRIX)
> lBrixMeans <- emmeans(lBrix, pairwise ~ Light : Variety, response = "type")
> cld(lBrixMeans)
```


Appendix III: Taste Test Instructions

Name _____

Terms to Use

Color Use these terms to generally describe appearance

- 1 Light Green
- 2 Dark Green
- 3 Mixed Red/Green
- 4 Red

Texture Use these terms to describe mouth-feel

- 1 Crisp
- 2 Crunchy
- 3 Soft

Taste Use these terms to describe the general taste

- 1 Bitter
- 2 Mild
- 3 Sweet

Taste Test Directions

1. Each station, marked with variety and “A” and “B”, contains a lettuce head grown under either LED or HPS.
2. Rip or cut off a piece of lettuce leaf from 1-3 inches in diameter or length. Pick a size that you feel will give you a good sense of taste and texture.
3. Chew the lettuce sample and record your thoughts in the appropriate box on your worksheet.
4. Either spit out your lettuce sample into a paper towel or the garbage.
5. Eat a pallet cleanser in between varieties (water and/or saltines).